THE

AMERICAN JOURNAL
OF THE MEDICAL SCIENCES

MARCH, 1942

ORIGINAL ARTICLES.

AUTO-HEMOLYSINIC ANEMIA WITH AUTO-AGGLUTINATION: IMPROVEMENT AFTER SPLENECTOMY.

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The acute hemolytic anemias may be classed in several groups according to etiology. Among the more obscure of these groups is auto-hemolysinic anemia, *e. g.*, acute hemolytic anemia due to the action of auto-hemolysins arising apparently spontaneously within the body. We have recently had opportunity to observe such a case, which presented, in addition to the criteria necessary for the diagnosis, certain other features of unusual interest, which it is the aim of this paper to discuss, along with a review of the literature on the subject of auto-agglutination.

Immunology and Biochemistry. Since there is considerable confusion concerning the nature of auto-agglutination and auto-hemolysis, a few definitions and general remarks are in order. Hetero-agglutination is the clumping of the blood cells of one species by the serum of another species; iso-agglutination, the clumping by the serum of a different individual of the same species; auto-agglutination, the clumping of an individual's cells by his own serum. The cause of the confusion is that the auto-agglutinins when they occur in human serum act, not only on the individual's own blood, but also on all other human blood so that they are really pan-agglutinins. Since they act on the blood of individuals other than the person from whom they are derived they are confused with iso-agglutinins. However, that the action on the individual's cells and other red cells

as well, is due to a single agglutinin, is shown by the observation that the agglutinin can be absorbed out, not only by the patient's cells but by any human blood cells.

The reaction of auto-agglutination is accelerated by cold and retarded by heating and in most cases is reversible at 37° C. It is not to be confused with the exaggerated rouleau formation and clumping—pseudo-agglutination, observed in acute infections, or hyperproteinemia where the erythrocyte sedimentation rate is accelerated.[19,24a] It is a true antibody reaction and it can be demonstrated that the antibody is adsorbed by the cells.

The source of auto-agglutinins is not clear. Landsteiner and Levine believe that auto-agglutinins acting only at low temperatures exist in most normal human sera and that under certain conditions their titer may become high enough to operate at higher temperatures.[24b] As pointed out above, these are non-specific agglutinins. What conditions may cause such a rise in the normal auto-agglutinin titer? Some light can be thrown on this question by reviewing the conditions in which auto-agglutination has been observed. A peculiar type of auto-agglutination and auto-hemolysis occurs in paroxysmal hemoglobinuria sometimes encountered in congenital lues. Auto-agglutination has been observed, frequently in hemolytic icterus, in trypanosomiasis, severe anemias, occasionally in pneumonia and even in apparently normal individuals. More recently it has been reported in hemolytic anemia following sulfanilamide therapy[1] and acute hemolytic anemia due to lead poisoning.[9]

In discussing the pathogenesis of acute hemolytic icterus, Dameshek and Schwartz[5] attribute the disease to the spontaneous formation of exceptionally potent auto-hemolysins and therefore believe that this disease in many instances is a hemolysinic anemia. They offer no explanation for this sudden formation of auto-antibodies. According to Wiener,[24d] on the other hand, the auto-antibodies are a symptom and a result of the disease rather than the cause. He believes that under certain conditions which may bring about a sudden massive destruction of the patient's own blood cells, these cells can act as an antigen and stimulate the formation of auto-antibodies, just as occasionally, transfusions of Rh+ blood into Rh− patients may stimulate the formation of anti-Rh iso-antibodies.[25] When only a small amount of hemolysis occurs the reticulo-endothelial system can take care of the effete cells, but when large amounts of blood are destroyed the accessory mechanisms of auto-antibody formation are brought into play. However, this process sometimes initiates a vicious circle since the auto-antibodies may dispose not merely of the effete cells, but hemolyze healthy cells as well, and these newly destroyed cells act as a stimulus to more antibody formation. A process similar to this may have taken place in the case herein reported. A similar concept was advanced by Guillain and La Roche in 1909,[11] who described the occurrence

in serum and spinal fluid of auto-agglutinins and auto-hemolysins following post-traumatic bleeding into the meninges.*

According to the unitarian hypothesis, hemolysis and agglutination are due to the action of the same antibody under different conditions. This is indicated by adsorption experiments in which agglutinin and hemolysin are removed together. There is also a correlation between the titer of hemolysin and agglutinin.[24c] Hasser[12] showed that if old cells were used normal iso-hemolysins for each blood group could be demonstrated almost as easily as iso-agglutinins. Therefore, we believe that although they may not always be found without special examination, auto-agglutinins are likely to occur in most cases of auto-hemolysinic anemia, and conversely, the presence of auto-agglutination in such cases may be so marked as to interfere with the satisfactory *in vitro* demonstration of auto-hemolysins. We believe it more satisfactory to regard these anemias as being due to an auto-antibody reaction.

Efforts have been made by some authors to explain auto-agglutination on the basis of alterations in the chemistry of the blood, particularly with reference to the blood proteins. Thus, this phenomenon is noted as occurring with great frequency in acute infections, multiple myeloma[19] and other conditions. However, as pointed out above, in these conditions the sedimentation rate is greatly accelerated and the red cells have an exaggerated tendency to rouleaux formation, and it is our belief that these instances represent pseudo-agglutination and not true auto-agglutination.

Review of the Literature. Following the discovery of Ehrlich and Morgenroth that a specific antibody reaction could be produced by inoculating a goat with red blood cells from another goat, and the studies on the mechanism of antibody reactions by the same authors, Bordet, Widal, and others, the way was cleared for the recognition of similar and allied phenomena in man. Experimental efforts to produce hemolytic anemias in animals were accompanied by the search to discover cases in man in which hemolysins and hemo-agglutinins could be recognized as being related to clinical anemia. Although the classification by Landsteiner of the normal iso-agglutinins in human blood was made in 1901,[14] this work did not receive wide notice until it was repeated by Jansky in 1907 and Moss in 1910. The cases reported by observers prior to 1910 as representing auto-hemolysis and auto-agglutination are, in many instances, either obviously cases of iso-antibody reactions or else open to strong doubt.

* In addition, it appears possible that there may also arise specific auto-agglutinins in view of the antigenic properties of red blood corpuscles. Koepplin reported 2 cases with auto-agglutinins, M with pernicious anemia and H with paroxysmal hemoglobinuria and hemolytic anemia.[13b] Anti-M's red blood cell dog serum was shown to agglutinate only M's and H's red blood cells and no other human red cells. Similarly, anti-H's red cell serum acted only on H's and M's cells. Furthermore, after having agglutinated either one's cells, such sera lost the power to act on the other's. As yet, no one has confirmed Koepplin's work.

The earliest instance that we can find reported in man was in 1890, but it is not well substantiated.[9] The first case to be described of an auto-hemolysin which was related to an acute hemolytic anemia in man was reported by Chauffard and Vincent in 1909.[4] An exhaustive review of the reported cases since then has been made by Dameshek and Schwartz in the past year.[5] In 1908 Widal, Abramé and Brulé,[23a,b] discussing the differential diagnosis of congenital and acquired hemolytic icterus, stated that the latter was characterized by the presence of auto-agglutinins which were not present in the former, quoted 4 cases, and later described a fifth.[23c] It is noteworthy that very few other authors of this time reported auto-agglutinins, although several reported auto-hemolysins. Several cases of auto-agglutinins then reported appear to us to be more likely iso-agglutinins and have therefore been omitted from this review.

Since then several cases of auto-agglutination in severe anemias due to various causes have been reported. Roth reported 1 case with pernicious anemia.[20] Boxwell and Bigger[3] reviewing the problem reported 22 cases in a search of the literature up to 1931, of which 11 suffered from severe anemias, and some of these had syndromes similar to an acute hemolytic anemia. In 1935 Koepplin[13] reported a case of auto-agglutination with pernicious anemia and another with acute hemolytic anemia[14] and also reviewed the literature reporting 15 additional cases. We have discovered reports of cases in the recent literature by Masters *et al.*[16] (2 cases), Patterson and Smith,[17] Giordano and Blum,[8] Watson[22] (2 cases), Greenwald,[10] Antopol, Applebaum and Goldman[1] (2 cases following sulfanilamide); Gray, Greenfield and Lederer[9] reported a case following several transfusions for an acute hemolytic anemia due to lead poisoning. Avoiding duplication where possible, this makes a total of 54 cases of auto-agglutination in an extensive (though by no means complete) search of the literature. Undoubtedly this phenomenon is overlooked in many cases, unless its presence is revealed by difficulty in cross-matching or some transfusion accident.

Case Report. This was the first admission of a 43-year-old Chinese restaurant cashier. The family history was negative for anemia or icterus as far as could be ascertained. The past history was negative. The present illness began about a year before admission with migratory polyarthritis of the fingers, hands, shoulders, knees and feet. In June, 1940, the patient sought medical attention and was admitted to the Hospital for Joint Diseases by Dr. B. The positive findings at this time were mild icterus (icteric index 16) and severe anemia with 1.50 million R.B.C. There were hemorrhages noted in the fundi and a palpable spleen. The urine showed numerous red cells and was positive for urobilinogen in 1:60 titer. Four transfusions from carefully matched professional donors were given following each of which he experienced a severe thermal reaction with increased anemia, deepening icterus and gross hematuria. An atypical agglutinin acting on blood of Groups O and A at 20° C. was demonstrated in his blood by Dr. Eugene Katzin and Dr. Philip Levine of the Blood Donor's Betterment Bureau. He had a fever as high as 101.4° F. The erythrocyte count

ranged from 1.50 to 1.31 million, hemoglobin from 7.5 to 3.8 gm. (Sahli). Three blood cultures were negative. An erythrocyte fragility test was not satisfactory due to strong auto-agglutination of the red cells. The blood cholesterol was 156 mg. per 100 cc., cholesterol esters 98 mg. per 100 cc.; the serum proteins were normal; serum calcium 8.7 mg. per 100 cc., phosphorus 4.4 mg. per 100 cc. A bone marrow biopsy showed a hyperplastic marrow filled with erythrocytes and normoblasts. In the middle of August the patient came under the care of one of us (M. K.) and was transferred to this hospital where the studies were continued.

Upon admission to Bellevue the patient's temperature was 100.2° F., pulse 98, respirations 20, blood pressure 130/60. He appeared chronically ill, obese, pale, and slightly icteric. The positive physical findings included slight puffiness of the eyelids, icteric scleræ, and many irregularly shaped hemorrhages with old patches of retinitis throughout the fundi. There was a well-healed biopsy wound over the sternum. The heart was not enlarged to percussion and there was a rapid regular rate and a soft apical systolic murmur. The abdomen was obese and non-tender with the tip of the spleen palpable. Rectal examination revealed a large solitary internal hemorrhoid. The remainder of the physical examination was essentially normal.

Laboratory. The blood count on admission was 1.35 million R.B.C. with 22% H . (All hemoglobin values hereafter are Sahli determination—16 gm. equals 100%.) White blood cells were 5600 with neutrophils 78% (Schilling count 0–16–62), lymphocytes 16%, eosinophils 6%, normoblasts 6/100 white cells, erythroblasts 3/100 white cells. There was marked hypochromia, aniso and poikilocytosis with great destruction of red cells in the smear. Many microcytes, some with hyperchromic centers, and macrocytes, Cabot rings, and polychromatic cells were present. The white cells were of normal size, shape and staining. Platelets appeared normal. No parasites were observed.

Further blood studies showed no change in the essential character of the smear. The hematocrit was 10.5%; mean cell volume 70 c.μ; reticulocytes 24%; platelets 272,800. The bleeding time was 4 minutes, clotting time 21 minutes, with marked clot retraction ½ hour after clotting. The prothrombin time (Smith modification of the method of Quick) was 5 minutes 2 seconds; a normal control was 2 minutes 50 seconds. The erythrocyte sedimentation rate (Wintrobe) was 80 mm. in 5 minutes, but the anemia was so profound that it was not possible to correct for it. The rate was undoubtedly affected by the marked auto-agglutination. Erythrocyte fragility was tested: the bloods of the patient and a normal control were both oxalated, centrifuged, the cells separated, washed in saline, and then centrifuged and resuspended in a 1:5 dilution of saline before being put to the test. Hemolysis began at 0.38 and was complete at 0.34 after 12 hours' incubation. The control values were 0.44 and 0.36 respectively. Clumping of the patient's cells was so marked that it is doubtful if a sufficiently good interface was obtained to allow adequate hemolysis in the higher tubes. Price-Jones curves done by measuring in succession the diameters of 300 cells on each smear showed a biphasic curve with a major peak at 6.8 μ and a minor peak at 8.5 μ. There was a marked broadening of the curve at the lower end of the scale indicating many microcytes.

Blood chemical findings were: NPN 35 mg. per 100 cc.; uric acid 5 mg. per 100 cc.; creatinine 1.3 mg. per 100 cc.; cholesterol 160 mg. per 100 cc.; albumin 3.8 mg. per 100 cc.; globulin 1.8 mg. per 100 cc.; icteric index 18; direct van den Bergh delayed, indirect positive; phosphatase 4.3 Bodansky units; Wassermann negative.

The urine was amber with sp. grav. 1.015; no albumin, glucose, acetone or bile; 2 to 4 red cells per high power field were visible.

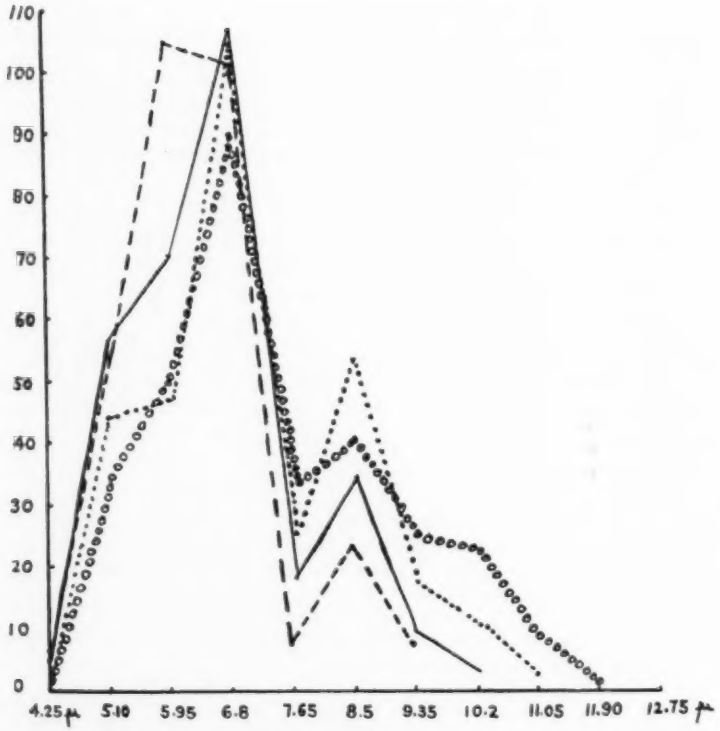

CHART 1.—Price-Jones curves of four different dates plotted on the same graph to illustrate the bi-phasic type of curve, and the shift in relative numbers of microcytes and macrocytes after splenectomy. (Each curve represents direct measurement of 300 consecutive cells on dried smears stained with Wright's stain.) (The curve made up of circles gives erythrocyte diameters 1 week after splenectomy.)

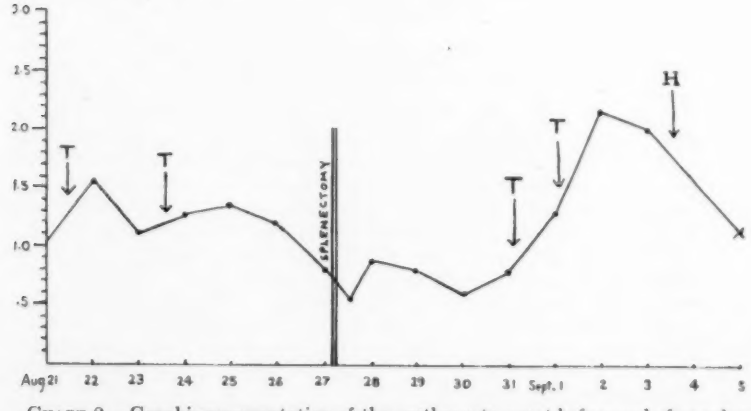

CHART 2.—Graphic representation of the erythrocyte count before and after splenectomy, with special reference to the effects of transfusion. H, indicates onset of rectal bleeding; T, indicates transfusion.

Gastric analysis revealed in the fasting specimen no free HCl, and only 3 units total HCl. One-half hour following histamine there were 37 units free HCl.

The electrocardiogram showed myocardial changes of the Q_2Q_3 type with inverted T_3 interpreted as being due to myocardial anoxia.

The Roentgen rays of the bones and joints of the hands, wrists and knees were interpreted as normal. A 6-foot chest plate revealed some old fibrotic nodules in the right apex and some thickening of the interlobar pleura between the upper and lower lobes on the right. The heart was moderately enlarged in all diameters.

Several examinations of the stools revealed no evidence of parasitic infestation.

Additional Blood Studies. Special hematologic studies were carried out in the Serologic Laboratory of the Office of the Chief Medical Examiner of New York under the supervision of Dr. A. S. Wiener. The tests were considerably hampered by the marked auto-agglutination of the patient's cells. After the blood had stood at room temperature for a few minutes the cells were strongly clumped and an even suspension in saline solution could not be obtained. In contrast to the usual case of auto-agglutination the clumping was not reversed by warming the suspension to body temperature, but an even suspension was finally obtained by washing the cells with saline at 50° C. Unfortunately, this treatment damaged the cells somewhat. The patient was found to be a Group A, sub-group A_1, type MN whose serum contained auto-agglutinins and also anti-O and anti-A_2 agglutinins, presumably, the result of a previous transfusion from a universal donor and a Group A_2 donor. It was impossible to separate the auto-agglutinins from the red blood cells by centrifuging, washing, resuspending in saline and heating in the usual fashion to body temperature. Because of the intensity of agglutination efforts to determine the presence of auto-hemolysins were unsuccessful, although it was observed that following incubation overnight, there did appear to have been some hemolysis. It was thought that, in addition to the auto-agglutinin and anti-O and anti-A_2 agglutinins there might also be antibodies against the so-called Rh factor recently described by Landsteiner and Wiener[15,25] but these could not be demonstrated.*

Further Course. From our blood bank 2 bottles of Group A_1 blood, Type M, which showed less tendency to agglutination than any others, were selected. Despite precautions of warming the blood before, and keeping the patient warm during the transfusion of 500 cc., he had a severe chill followed by a rise in temperature to 105.8° The red blood count rose from 1.02 million (22% Hb.) to 1.53 million (27% Hb.) the next day, but the day after that it had fallen to 1.09 million (25% Hb.). The patient was more deeply jaundiced and his blood showed no trace of the M cells. Another transfusion was given in the same manner with a reaction followed by a transient rise to 1.53 million (27% Hb.) and subsequent fall to 790,000 (20% Hb.) and again complete disappearance of the donor's cells. There was no evidence of hemoglobinuria following either transfusion. After the second transfusion it was felt that immediate splenectomy to remove the chief source of blood destruction was urgently indicated.

* In retrospect, despite the failure to demonstrate anti-Rh antibodies in the patient's serum, Wiener believes that the possibility is not excluded, and that the Rh factor may have played a part in the transfusion reactions. Because of the difficulty in obtaining a good cell suspension it could not be ascertained whether the patient was Rh-minus or Rh-plus. Moreover, it has been found that transfusion reactions due to the Rh-factor can occur in the absence of demonstrable Rh iso-antibodies in the patient's serum.[24e] In the future when confronted with the same problem we would advise the routine use of Rh-minus donors as an additional safeguard.

On August 27 the patient's spleen was removed together with a small section of the liver for biopsy by Dr. John Morris and Dr. Lester Breidenbach (Fourth Surgical Division). Plasma (250 cc. Type A) was given during the operation and the patient was returned to the ward in good condition. The erythrocyte count postoperatively was 550,000 (15% Hb.); the next day it was 880,000 (22% Hb.).

The spleen weighed 400 gm. The cut section was firm and deep red. The microscopic section showed great congestion of the pulp, with numerous islands of extramedullary hematopoiesis. The liver section showed normal hepatic architecture with islands of extramedullary hematopoiesis and periportal infiltration of round cells and occasional eosinophils.

The course postoperatively was uneventful until the third day when the patient developed a pneumococcus Type I lobar pneumonia in the left lower lobe. Sodium sulfapyridine was given intravenously with prompt fall in temperature and striking improvement within 12 hours. Studies of the blood 4 days following operation showed that the auto-agglutinin titer was still high, but in view of the desperate situation it was decided to hazard a transfusion on the chance that there would no longer be a reaction. A professional donor of Group A_1 was selected and cross-matched and a transfusion of 500 cc. diluted with saline was given in fractionated amounts with no reaction on August 30. The red count rose on September 1 to 1.28 million (34% Hb.) and many normoblasts appeared in the smear. On that day a second donor was used in the same manner with no reaction, and on September 2 the red count was 2.12 million (48% Hb.) and on September 3, 2.03 million (34% Hb.), which indicated that the blood destruction had practically ceased. The auto-agglutinins persisted as before despite the absence of reaction. Normoblasts at this time were 121/100 white cells and this was reflected in the Price-Jones curve which showed a falling off of the number of microcytes and increase in the macrocytic cells. On September 3 the temperature rose and the patient began to complain of abdominal pain and to pass large amounts of chocolate-colored blood by rectum. The white count rose to 39,300 with 98% neutrophils, and following the onset of bleeding the red blood cells fell very rapidly, to 1.13 million (26% Hb.) on September 4. The patient continued to bleed and expired on the ninth postoperative day. Throughout the whole postoperative course there was a tendency to edema which was counteracted by plasma transfusions every few days. Unfortunately, permission for a necropsy could not be obtained.

Discussion. This case fulfilled the criteria usually accepted for the diagnosis of acute hemolytic anemia. The patient showed icterus, anemia, hemoglobinuria at least once at the other hospital, microcytosis and marked evidence of new blood formation with reticulocytosis. The biphasic Price-Jones curve has been emphasized by Dameshek and Schwartz[5] and demonstrated experimentally by Tiggert[21] *et al.* as being characteristic of the hemolytic anemias. The question may be raised whether this may have been a case of familial spherocytic jaundice. Both spherical microcytes, and increased erythrocyte fragility have been demonstrated repeatedly in other types of hemolytic anemia[2,5,7,18,19,21] (clinical and experimental) and there was a negative family history in our case. As far as we could determine there was no evidence of parasitic infestation, or exposure to any toxic agents which could cause an acute hemolytic anemia. Therefore, we regard this as a case of acute hemolytic

anemia due to the spontaneous action of some intrinsic factor, in this case an auto-antibody.

We have stressed the inter-relationship between hemolysins and agglutinins, and we believe that there was a strong auto-hemolysin active in this case, although it was impossible to demonstrate it *in vitro* due to the overwhelming presence of an auto-agglutinin. An unusual feature of this auto-agglutinin was the fact that its action persisted at body temperature and that it could not be split off from the red cells by any methods tried. To the best of our knowledge this is an entirely unique phenomenon which we are unable to explain. The unusually high titer of the agglutinin was probably due to repeated transfusion reactions with hemolysis of blood and liberation of more antigen. The anti-O and anti-A_2 agglutinins demonstrated did not play any further clinical rôle, since the patient received no further transfusion with other than A_1 blood. They probably represent, along with the auto-agglutinin, a heightened capacity of the patient's antibody forming elements to respond to antigenic substance.

The factor which precipitated the initial hemolysis remains unknown, but a vicious circle was established, hemolysis necessitating transfusions which in turn led to increased hemolysis. The only treatment which could break this circle was the removal of the chief site of blood destruction, the spleen.[6] That this rationale was correct was indicated by the fact that following splenectomy the patient was able to receive transfusions without reaction in spite of the persistence of the high auto-agglutinin titer. This also suggests strongly that the spleen itself was the actual site of hemolysis of the cells on which the hemolysin was adsorbed.

It is a striking testimony to the efficiency of the drug therapy of pneumonia that the patient recovered from his postoperative infection. We had some hesitation about giving the drug in view of its occasional hemolytic tendencies,[1] but we had no other choice because the result of typing was not immediately available. In spite of the drug the red blood count rose until the terminal bleeding episode began. The cause of death is unproven, but the evidence suggests it was due to exsanguination from hemorrhage into the gastro-intestinal tract.

Summary. 1. The problem of acute hemolytic anemia due to autohemolysins is discussed with particular emphasis on the phenomenon of auto-agglutination.

2. A review of the literature reveals 54 cases of auto-agglutination. The immunologic aspects of autohemolysis and auto-agglutination are discussed and the more satisfactory concept of the auto-antibody reaction is advocated.

3. A case of acute hemolysinic anemia with a strong auto-agglutinin is described. This auto-agglutinin was unique in that it persisted despite warming to 37° C. and could not be separated from the red blood cells.

4. In the presence of the spleen the auto-antibodies caused the destruction of blood cells introduced from without, but following splenectomy, transfusions were tolerated with rise in the blood count, despite the persistence of the auto-antibodies. It would appear from this that splenectomy is the rational therapy in such cases.

The authors wish to express their appreciation to Dr. A. S. Wiener of the Serological Laboratories of the Chief Medical Examiner's Office of New York City for examining the blood of the patient, and for his advice with regard to the preparation of this article.

We are indebted to Dr. J. G. M. Bullowa who treated this patient at the Hospital for Joint Diseases for allowing us to report this case and for the extensive laboratory data assembled prior to his admission to Bellevue Hospital.

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CHRONIC LEUKEMIA IN THREE SISTERS.

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The following cases of leukemia are of interest because of the occurrence of this disease in 3 sisters, and in an unusual combination, 2 of them clearly being of the lymphatic and 1 clearly of the myelogenous type.

Although familial leukemia has been described a number of times,[2,3,5,7–9] most of the reports concern but 2 members of a family. There are only a few descriptions of 3 or more cases in one family. McGavran[6] reported 3 cases of leukemia in one family: 2 brothers, 1 of whom had myelogenous leukemia and the other lymphatic

leukemia, and an uncle on their father's side who had the lymphatic type. Decastello[4] reports an interesting family, observed by Weiss and himself, 6 members of which had lymphatic leukemia within two generations. Out of 5 brothers and sisters of the first generation, 3 died of leukemia. Out of 9 members of the next generation, 3 died of leukemia, all of them were children of 2 of the 3 members of the previous generation who had died of the disease. Boggian[1] observed a family of 2 brothers and a sister in which 1 brother had 9 children and 3 of them developed lymphatic leukemia, and the sister had 3 children, 1 of which had lymphatic leukemia.

There is insufficient evidence at the present time to explain the occurrence of familial leukemia on the basis of heredity, except for the known heredity factor in cancer, of which leukemia is now usually regarded as an example. However, in view of reports of different combinations of blood diseases in the same family, as well as leukemia, it would seem that some predisposition to abnormality of the blood-forming tissues does exist in certain families. In reviewing the literature it is interesting to note that most of the cases of leukemia occurring in more than one member of a family are of the lymphatic type.

CASE 1.—A white female, who developed purpuric spots over the legs and occasional oozing of blood from the nose in the summer of 1927, when she was 39 years old. In October, 1927, and February, 1928, she had severe bleeding from the nose and mouth and developed purpuric spots over the entire body. These cleared up from time to time but she noted a tendency to bruise easily. She was hospitalized in April, 1928, because of earache, and examination at that time revealed the following: rapid pulse, afebrile, moderate degree of pallor, enlarged lymph glands in the cervical, supraclavicular, axillary and epitrochlear regions, spleen questionably palpable, no purpura at that time, heart and lungs normal. Blood studies revealed: erythrocytes, 3.69; hemoglobin, 80%; leukocytes, 18,500. Differential: neutrophils, 36%; lymphocytes, 64%. A re-check showed: leukocytes, 18,000. Differential: neutrophils, 30%; lymphocytes, 70%. A blood culture was sterile.

She continued to have hemorrhages from the nose and mouth and into the skin, and died in August, 1928, at the age of 40. While postmortem examination was not done, there was no question of the diagnosis of chronic lymphatic leukemia in the mind of the attending physician, who had observed her closely throughout the illness.

CASE 2.—A sister of the previous patient developed general weakness and swelling of the neck glands in the latter part of 1934, at the age of 55. In February, 1935, a blood study revealed: erythrocytes, 3.95; hemoglobin, 68%; leukocytes, 36,800. Differential: neutrophils, 20%; lymphocytes, 80%. Her course continued unfavorably with increasing loss of strength and weight and gradual development of general glandular enlargement. A blood study late in 1935 was said to be about the same as that above. In March, 1936, she was sent to a Baltimore hospital for Roentgen therapy. Examination at that time revealed a poorly nourished female with generalized glandular enlargement. The abdomen was tender throughout and there was a question of free fluid being present. The liver and spleen could not be palpated satisfactorily because of tenderness. No mention of purpura was made. Blood studies on March 23, 1936, revealed: erythrocytes,

3.90; hemoglobin, 76% (Sahli); leukocytes, 153,000. Differential: neutrophils, 2%; lymphocytes, 95%; pathologic lymphocytes, 3%. Another study 5 days later showed: leukocytes, 190,000, with a differential count of 1% neutrophils and 99% lymphocytes. Diagnosis: chronic lymphatic leukemia.

She was given Roentgen therapy over the spleen, inguinal and axillary glands, but her condition became suddenly worse and she died, March 30, 1936, at the age of 56. No autopsy was done.

CASE 3.—A sister of the previous 2 patients was first seen in August, 1939, at the age of 55, with symptoms of 1 year's duration consisting of undue fatigue, blue spots on the body, and loss of 18 pounds in weight. Examination at that time revealed a rather poorly nourished female with a fever of 99.6° F., pulse rate 120, blood pressure 170/100. One small hemorrhage was present in the fundus of the right eye and there were several large ecchymoses on the lower extremities but no fine petechia. No glands were palpable. The spleen was felt at the level of the umbilicus. The remainder of the physical examination was negative. A blood study showed: erythrocytes, 2.57; hemoglobin, 68% (Haden-Hausser); leukocytes, 162,000. Differential: neutrophils, 57%; lymphocytes, 2%; myelocytes, 30%; myeloblasts, 8.5%. She was sent to a Baltimore hospital for Roentgen therapy and was given a total of 796 r units over the spleen from September 21 to September 27, 1939. Blood counts on admission, prior to therapy, were practically the same as those given above. However, additional blood studies were done and are as follows: bleeding time, 2 minutes; clotting time, 3 minutes, 20 seconds; volume index, 1.10; M.C.V., 96.8 c.μ; reticulocytes, 3.2%; peroxidase stain showed 96% granular cells and 4% nongranular; platelets, 318,000; nucleated reds, 2/200.

Ten days after the last Roentgen ray treatment had been given the blood picture was as follows: leukocytes, 11,500. Differential: neutrophils, 60%; lymphocytes, 19%; monocytes, 2%; eosinophils, 10%; basophils, 8%; myelocytes, 1%.

She improved clinically following Roentgen therapy; the spleen was no longer palpable, the ecchymoses disappeared and she felt stronger generally. Through the winter of 1939–1940 her general condition remained reasonably good, although blood counts at frequent intervals revealed a gradual rise in the total leukocytes with increasing numbers of immature myeloid cells. In June, 1940, she was given further Roentgen therapy over the spleen, which had enlarged to 3 cm. below the costal margin. A blood study previous to this treatment was as follows: erythrocytes, 3.72; hemoglobin, 72% (Sahli); leukocytes, 137,500. Differential: neutrophils, 73%; lymphocytes, 1%; monocytes, 1%; eosinophils, 8%; myelocytes, 15%; promyelocytes, 1%. Following treatment the leukocytes dropped gradually to 5700 in July, 1940, and only a few immature cells were noted in the smears.

During the remainder of 1940 her condition was satisfactory except for a bout of low back pain apparently brought on by heavy lifting. Roentgen rays of the spine were interpreted as showing decalcification of the bodies of the lumbar vertebræ but no actual bone destruction or deposits of any kind. The condition improved under appropriate treatment.

Late in December, 1940, the spleen was again palpable to the level of the umbilicus and the liver edge was felt 5 cm. below the costal margin. No glandular enlargement was noted. The blood study showed: leukocytes, 138,000. Differential: neutrophils, 51%; lymphocytes, 2%; monocytes, 1%; eosinophils, 3%; basophils, 5%; myelocytes, 17%; myeloblasts, 4%; metamyelocytes, 17%. In February, 1941, she lost 10 pounds in the space of 3 weeks and developed many ecchymoses over the body and extremities. In March, 1941, she was given further Roentgen therapy, receiving 4 treat-

ments over both the liver and spleen. At the time of the last examination in May, 1941, she felt well generally, the spleen was not palpable, no glandular enlargement was noted and the blood count showed 6650 leukocytes with a practically normal differential ratio. No immature cells were present. Diagnosis: chronic myeloid leukemia.

The remainder of the family history is as follows: the mother died at the age of 73 of heart trouble; the father died at the age of 63 following accidental injury. One sister is living and well and blood counts during the course of a hospital admission in 1939 were normal. Two brothers are living; 1 in good health, and the other suffering from hypertension and stomach trouble. While blood studies are not available on either one there is apparently no evidence of a blood dyscrasia. Case 3 has 2 daughters who are living and well and recent blood studies on both were normal. There was no question of a blood disorder in any of the grandparents, as far as is known.

Summary. An instance of familial leukemia is reported in which 3 sisters have been affected, 2 with the chronic lymphatic type and 1 with chronic myeloid leukemia.

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THE NORMAL PLASMA COAGULATION TIME.*

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The coagulation time of recalcified oxalated blood plasma has recently been reintroduced as an important clinical laboratory procedure. It appears to be serviceable as a simple test for vitamin K deficiency.[1a,4] This test is a modification of "Howell's prothrombin time," which does not measure prothrombin, and it is less complicated to perform than the prothrombin determinations which are now in use. Although the simplicity of this test makes it available for general practice, it must be appreciated that the standardization of the technique of the procedure is on a purely empirical basis, as are all other such procedures, and that the reactions obtained do not duplicate normal physiologic processes.

* This work was supported in part by a grant from the Medical Research Council of the American Medical Association and in part by a grant from the Eli Lilly and Company.

The practical general application of a test depends not only upon its accuracy, but upon its simplicity. If it can be shown to be reasonably accurate and serviceable under a given set of conditions, it may justly be used as a diagnostic procedure. In order to meet these qualifications, tests of plasma clotting time must give constant readings in a large group of normal individuals and the physical conditions of the experiment as carried out must not materially alter the results obtained. With this in mind, tests have been performed on the plasmas of 340 normal adults and on 107 patients with non-hemorrhagic disorders. The effect of the technique of venepuncture, the amount of oxalate and calcium added to the blood and plasma, the method of collecting the plasma, and the effect of temperature have been noted in their relationship to the results of the procedure as outlined.

Technique of Test. The test of plasma coagulation time is routinely carried out as follows:

1. A clean venepuncture must be performed, obtaining the required amount of blood in a dry syringe.

2. The blood must be rapidly transferred to a test tube containing a weighed amount of dry potassium oxalate. A number of these tubes may be prepared at one time by adding 2% potassium oxalate and evaporating the solution so that the tube will contain oxalate in the ratio of 10 mg. to 5 cc. of blood. Centrifuge tubes are usually prepared with 5 mg. of oxalate for 2.5 cc. of blood (0.25 cc. of the 2% solution per tube). The oxalate must be thoroughly mixed with the blood by inverting the corked tube 10 or 15 times.

3. The oxalated blood is centrifuged in a constant-speed centrifuge at 3500 r.p.m. for 5 minutes and the plasma pipetted off.

4. Two-tenths of a cubic centimeter of plasma is pipetted into each of 2 small chemically clean test tubes and 0.2 cc. of 0.4% $CaCl_2$ is added to the first tube and 0.1 cc. to the second. The tubes should be gently shaken 5 to 10 times to secure thorough mixing. They need not be inverted.

A liter of the 0.4% calcium solution may be made up at a time, using chemically pure $CaCl_2$.

5. The coagulation time of the plasma should be read in minutes. The end-point is taken when the solution no longer flows when the test tube is held horizontal. The shortest time of coagulation for the two tubes is the plasma coagulation time.

6. The room temperature at which the test is carried out should be recorded. A variation between 23° and 26° causes no gross variation in the test.

7. The test should be completed within the first hour after the venepuncture.

The Calcium Factor. As the test is carried out, sufficient calcium is added to produce the shortest clotting time for the amount of oxalated blood used. This has been determined for the particular technique involved as previously described.[1a] It is now well recognized that substitution of the same amount of calcium as has been combined by adding potassium oxalate is not satisfactory.[3] It is also well recognized that adding an excess of calcium prolongs the plasma time[5] and adding too little calcium also prolongs the coagulation time or prevents coagulation from occurring.

The Collection of Blood Plasma. Blood plasma may be obtained by allowing the blood to stand until a sufficient volume of cells is settled out to permit pipetting off clear plasma from the upper level of the tube contents. A more rapid and satisfactory method is to centrifuge the oxalated blood which clears the plasma of formed elements. Centrifuging only a few minutes at low speed fails to separate all the cells and platelets from the plasma, while centrifuging 30 minutes at high speed will produce almost a complete separation. As the coagulation time of the plasma depends partly on its content of formed and recently broken up platelets, the time and speed of centrifuging are important factors in standardizing any test of plasma-clotting time. The time and the speed chosen for the test are entirely arbitrary and consequently a constant time and speed must be utilized in order to obtain comparable results. The time and speed chosen were selected because they produce an almost complete sedimentation of cells in a brief period which can be accurately regulated in the Gyro constant-speed centrifuge.[6] The effect of variations in the speed of a centrifuge is shown in Table 1. The slower rates of 1200 and 1800 r.p.m. tend to shorten the coagulation time because of the higher concentration of thrombokinase; and conversely, the rapid rate of 3500 r.p.m. reduces this concentration and prolongs the time. Similarly, centrifuging at a given speed for a short time shows the same type of effect in coagulation. Such changes are also shown in Table 1.

TABLE 1.—COMPARISON OF SLOWER SPEEDS OF CENTRIFUGING FOR LONGER PERIODS OF TIME WITH THE STANDARD HIGH SPEED OF 3500 R.P.M. FOR 5 MINUTES.

Case No.	R.P.M.	Tube No.	Time Centrifuged. 5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	Control 5 min. at 3500 R.P.M.
1	1200	1				5		6	5
		2				4.5		5	5
2		1				6		8.5	5
		2				5		5	5
3		1				9		5	7
		2				6		5	5.5
4		1		7		7			7.5
		2		5.5		6			5.5
5		1		4		6			7.5
		2		4		5			6
6		1	5		4.5			7	8
		2	4		4.5			5.5	6
7	1800	1	4		4				4.5
		2	3		3				3.5
8		1	4		6.5			6	5
		2	3.5		5			6	4
9		1	5.5	6	6.5				5
		2	4	4	4.5				4
10		1	6.5	7.5	7.5				6.5
		2	5.5	5.5	5.5				5
11		1		5	6				6
		2		5	4				4
12		1	7.5	7	7.5				7.5
		2	6	6	7.5				7

Because a high-speed centrifuge may not always be available, lower speed centrifuging for a longer period of time has been carried out in 12 cases to ascertain whether or not comparable results may be secured. The results obtained as shown in Table 1 indicate that plasma coagulation times are comparable when the blood is centrifuged at 1200 r.p.m. for 30 minutes or at 1800 r.p.m. for 15 minutes, as they do not deviate more than 1 minute from the standards set up at 3500 r.p.m.

Nygaard[4] has obtained plasma for his test of clotting time by allowing oxalated blood to stand 4 hours at ice-box temperature and omitting centrifuging. His tests have been carried out at 37° temperature and his normal figures are considerably shorter than those obtained after centrifuging in the manner described for room temperature. However, 30 samples of plasma obtained in the usual way have been tested for coagulation at 37° as shown in the last column of Table 2. The times have varied between 1.5 and 3 minutes, averaging 2.18 minutes or 130.8 seconds. This average is slightly less than Nygaard's figure of 161 seconds for 57 normal cases read by coagelgram. These findings indicate that the plasma obtained in 5 minutes of centrifuging is grossly comparable to that obtained after 4 hours of settling out at ice-box temperature.

Variations in Temperature. It has long been known that variations in temperature have a marked effect on the clotting of blood and blood plasma. Cold prolongs the clotting time and heat up to a certain degree shortens it. Minot[2] showed in 1916 that through a range of temperature from 15° to 37° there was a gradual shortening of the time with each degree of temperature. Nygaard has recently shown the same effects with his method of testing plasma-clotting time.[4] He found that there was a shortening of the time up to 37° C. and that the time tended to become longer at higher temperatures. Consequently he chose 37° as the standard temperature for his test. For the technique of the test already outlined[1a] room temperature was chosen because it eliminated the use of constant temperature water baths which might not always be readily available and because variations at the usual room temperatures of 23° to 26° C. were sufficiently slight so as not to alter the interpretation of the test. It was readily recognized that the results of the test would be more accurate if run at a constant temperature. However, normal plasma run at room temperature fell within a range of 2 to 8 minutes when the temperature in the laboratory varied between the extremes of 19° and 26° C.

With these variations in mind, a series of 122 normal tests has been run at the laboratory temperatures between 19° and 26° and an additional 28 tests at 27°, 28°, and 29°, and normal standards set up for each temperature. These normal standards for the individual temperatures are presented in Table 2 and Chart 1. It is obvious that the temperature range from 23° to 28° produces very

little change in the rate of clotting, the average variations lying between 4.5 and 5.7 minutes. It is noteworthy that there is a sharp accelerating effect at 29° to 2.7 minutes, and there is a progressive prolongation of the coagulation time from 23° down to 19°, which corresponds to Minot's observations.[2]

TABLE 2.—VARIATIONS IN PLASMA COAGULATION TIMES AT DIFFERENT TEMPERATURES IN 180 CASES.

Temperature.	No. of cases.	Shortest time.	Longest time.	Average time.
19°	5	7	11	9
20°	13	5	10	8.2
21°	12	4.5	8	7.3
22°	18	5	8	6.6
23°	30	4	8	5.7
24°	20	4	7.5	5.2
25°	20	3	7	4.9
26°	4	4	7	4.8
27°	9	3	6	4.7
28°	8	3	6	4.5
29°	11	2	4	2.7
37°	30	1.5	3	2.18

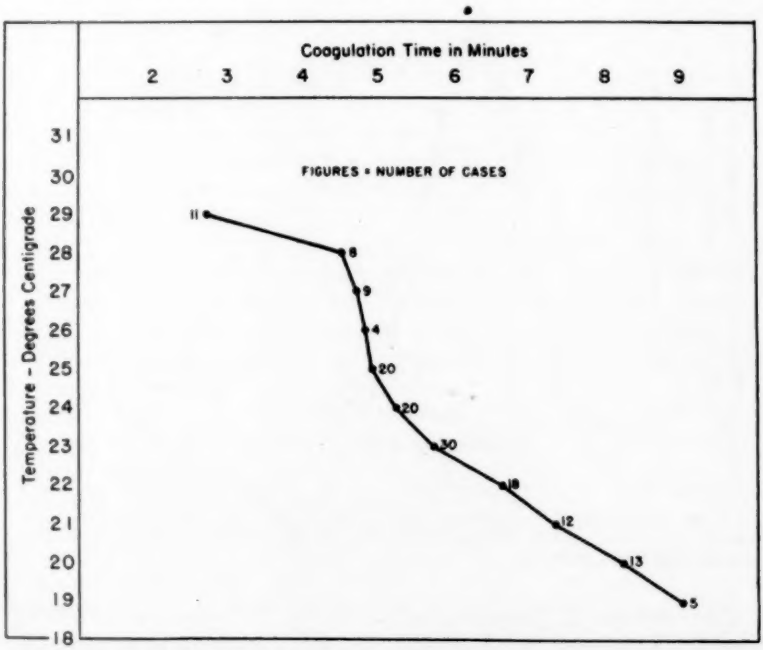

CHART 1.—Relationship of plasma coagulation time to temperature in 122 cases. Variation of less than a minute at the commonly encountered laboratory temperatures of 23° to 26°; progressive prolongation of time at lower temperatures and marked shortening of time at 29°.

In setting up the test at room temperature, it is necessary to appreciate that an apparently slight increase in plasma coagulation

time may be due to an unusually cool room atmosphere and that an apparently short time may be due to an increased temperature in the laboratory occurring in the summer or due to artificial heating. With this in mind, room temperature may be considered satisfactory in carrying out the test, particularly when it lies between 23° and 26°, as the clotting time for 74 cases in this range averaged 5.3 minutes which is to be compared to 5.25 minutes, the average normal time for 340 cases run at room temperature as shown in Chart 2. If a water bath is available to maintain an even temperature of 24°, the results will be more constant. The average time for the 20 cases run at 24° is 5.2 minutes.

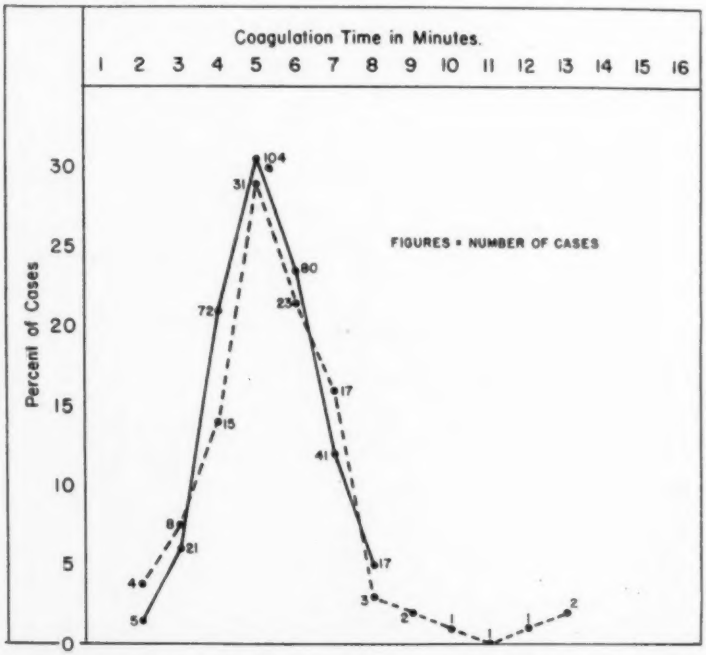

CHART 2.—Distribution of plasma coagulation time by minutes in 340 normal individuals (solid line) and in 107 patients with non-hemorrhagic disorders. Average normal time—5.25 minutes.

The Addition of Thromboplastin. In the test of plasma clotting time no thromboplastin is added. As this substance is an unstable tissue extract, its use complicates the test. It is added in tests of "prothrombin time" to assure an excess of thrombokinase, presuming that as a result a prolongation of the coagulation time will represent a quantitative prothrombin deficiency. This assumption does not take into consideration the concentration of anticoagulant substances in the blood.

The plasma coagulation time actually represents the resultant effect of the relationship of the clot-promoting substances and the clot-retarding substances in a given plasma under the conditions of the experiment. If these substances are out of balance due to prothrombin deficiency, a prolongation of the clotting time will occur and a tendency toward bleeding. However, if a similar deficiency of thromboplastin occurs, clotting time might be prolonged independent of a disturbance in vitamin K metabolism. Actually such a deficiency rarely occurs, and then in the writer's experience only in the presence of marked thrombocytopenia. Two patients out of 23 studied with purpura showed such a prolongation. Both had advanced leukemia with almost a complete absence of platelets from the blood and both showed only slight increases in coagulation time. Nygaard has observed similar cases,[4] but the clinical and laboratory features of these cases could hardly be confused with vitamin K deficiency, unless they occurred coincident with liver disease and jaundice.

Not only does the addition of thromboplastin appear to be unnecessary, but quantitative diminutions of prothrombin as shown by prolonged "prothrombin times" may not indicate a hemorrhagic tendency. This is well illustrated by the physiologic hypoprothrombinemia of the new-born.

Other Factors Which May Influence the Test. Up to the present time no study of the effect of age of the individual on the plasma coagulation time has been recorded. It is an impression of the writer that elderly people may tend to show a slightly shorter coagulation time than the average normal of 5.25 minutes. Also the possible effects of sex have not been studied statistically but no differences have been noted. However, in running tests of plasma coagulation time on the cord blood of 11 infants, it was found that the average time was only 2.7 minutes and that the limits of variation were between 2 and 3 minutes, except for one reading of 4.5 minutes. It is assumed that this hastening of coagulation was produced by an increase in thrombokinase of cord blood which may readily occur when the cord is cut. Seven observations on 3 infants in the first 3 days of life showed coagulation times from 3 to 6 minutes with an average of 4.4 when the blood was obtained readily by venepuncture. Only a few children have been studied and they showed no variation from normal adults.

Normal Variations. The plasma coagulation time has been performed on 340 normal individuals. This group includes the 100 cases previously reported.[1a] All the tests run have been incorporated in this series except those run in a water bath at less than 21° or greater than 26° for statistical purposes for Table 2 and Chart 1. The results are presented diagrammatically in Chart 2, which shows the number of cases and percentage incidence for the coagulation time in minutes from 1 to 13. Clotting occurs in 86.35% of the

cases in 4 to 7 minutes with an average for the whole group of 5.25 minutes. This is 0.13 minutes shorter than the normal average of 5.38 minutes already noted,[1a] but is evidently due to the cases being more evenly distributed throughout the slightly warmer summer months. The majority of the 17 cases with a clotting time of 8 minutes occurred in the winter at temperatures of 21° or below, and consequently this figure should be considered at the upper limits of normal. Table 2 shows that this reading did not occur above 23° and it was only encountered once in the 30 cases read at 23°.

The plasma coagulation time has also been studied in 107 patients with non-hemorrhagic types of disease. For the sake of comparison, the results in this series of cases have been compared to the normal in Chart 2. The trend of the curves is similar with few exceptions. The short time of 2 minutes is more than twice as common and may be due to the inclusion of 3 cases of venous thrombosis in this group. The lower incidence of 4-minute times is unexplained. The appearance of prolonged coagulation times above the normal upper limit of 8 minutes in 6 cases is worthy of comment.

Of the 2 patients with times of 9 minutes, 1 was suffering from prolonged malnutrition and the other was receiving sulfanilamide therapy for a streptococcus sore throat and fever of 39°. The patient with a 10-minute time suffered from severe allergy, and the one with the 12-minute time had a marked chlorotic achlorhydric anemia. The 2 patients with the 13-minute times had disorders similar to the first 2 patients. It is evident that an occasional patient may have a disturbance in plasma coagulation without known liver or biliary tract disease, hemophilia, or purpura which are the recognized disorders which may cause abnormal tests. Such a condition associated with malnutrition and relieved by vitamin K therapy has already been reported.[1b]

Summary. 1. The plasma coagulation time is a satisfactory test for vitamin K deficiency, as previously noted.

2. The test has been carried out by a standardized technique in 340 normal cases and in 107 with non-hemorrhagic conditions.

3. The importance of a constant speed and time in centrifuging the blood has been emphasized.

4. The effect of changes in temperature upon the results of the test have been pointed out. Room temperature is satisfactory under ordinary conditions.

5. Standards have been set up for laboratory temperatures which may be encountered and for 37° C.

6. The constancy of the results obtained warrants the use of the plasma coagulation time as a simple laboratory test, provided the physical conditions of the procedure are closely adhered to.

The writer is indebted to Miss Thelma Olsen and Miss Elissa Addis for their technical assistance in carrying out the laboratory tests.

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THE EFFECT OF PARITY ON THE AVERAGE BLOOD PRESSURE AND ON THE INCIDENCE OF HYPERTENSION.

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Much has been written concerning the incidence of hypertension and renal and vascular lesions[2,4,10] in patients whose pregnancies have been complicated by albuminuria and hypertension. The belief that the "toxemias of pregnancy" play a part in the production of these changes has become firmly entrenched in medical thought and literature.

Although the high incidence of patients who have hypertension 5 to 10 years following "toxemic" pregnancies has been stressed, no such "follow-up" studies are available on women who have had pregnancies not complicated by evidence of vascular and renal abnormalities.

Several reports[1,3,11] in the literature have expressed the belief that the "toxemia" syndrome occurs in those patients who otherwise would become hypertensive, but little real evidence has been brought forward to substantiate this impression.

Studies of blood pressure curves for men and women show that both the systolic and diastolic pressures for women exceed those of men after middle age has been reached. It occurred to us that this significantly higher level of systemic pressure might possibly be explained by the inclusion of a certain number of women whose vascular systems have sustained injury during toxemic pregnancies.

The present communication attempts to determine the incidence of hypertension and the average blood pressure in women of varying age groups who have and who have not sustained pregnancies and to determine how such findings can be related to the toxemias of pregnancy.

Materials and Methods. From the case histories of 1800 women who were admitted to the Gynecological service of the Woman's Clinic as in-patients during the years 1933–1940, there were selected 900 records of nulliparous women* and 900 of parous women. These cases were chosen by sequence of history numbers to satisfy the age group quota. Both parous and nulliparous groups were subdivided according to age by decades from 20–29 to 60 or more; each of the age groups contained 200 cases except the groups including patients 60 or more years of age, and here there were 100 cases.

The blood pressure recorded for each case used in this study was obtained by averaging pressures recorded in the history in the same period of each patient's hospital stay and under similar conditions, and usually consisted of: the pressure determined at the clinic visit; the pressure taken in the admission examination to the hospital; the pressure recorded by the anesthetist prior to administration of anesthesia; and pressures from two subsequent visits to the hospital, either in the clinic or on admission. No pressure determinations were included unless they were taken by a member of the interne staff. Cases were discarded where organic disease of the aortic valve, fever, cardiac tamponade, or general debility were present.

In 90% of our cases more than 1 blood pressure was recorded; 60% had 3 or more; and in 45% pressures were taken at different visits to the hospital, some even in different years.

Only those patients were considered hypertensive whose *averaged* systolic pressures were 140 mm. Hg or more *and* whose *averaged* diastolic pressures were 90 mm. Hg or more. By this method only those patients with persistent elevation of both systolic and diastolic pressures were considered as hypertensive.

Blood pressure determinations were made on all patients by the auscultation method while in recumbent position. The systolic pressure was taken as the point at which the sound first appeared while the cuff was being slowly deflated, the cuff being originally inflated to a level exceeding this figure. The diastolic pressure was considered at the level at which a sharp demarcation of sound occurred prior to the disappearance while deflating the cuff (the beginning of the fourth phase).

An attempt was made to approximate the age, color, and weight of the patient and the type of operation to which she was subjected. Parity was determined by the number of total pregnancies experienced *less* the number of pregnancies interrupted prior to viability of the child. In this manner no pregnancy interrupted prior to the seventh month was considered to produce parity.

Most patients were weighed on admission to the hospital.

Table 1 shows the averaged systolic and diastolic blood pressures for both the parous and nulliparous women according to age groups.

It is seen here that a slightly higher systolic level is maintained by the parous woman when compared with the nulliparous at all ages save the 20–29 age group where there is a slightly lower level. There is no consistent difference in the diastolic level save in the 30–39 group, where the parous group shows a minimally higher level (Chart 1).

It is of interest that the incidence of hypertension, given in Table 1, shows a slight increase among the nulliparous patients

* Since it was impossible to get a complete series of records for nulliparous women in the 50 to 59 and 60 or more age groups from the Gynecological Service, it was necessary to add to these groups a few records of cases admitted on the Medical and and Surgical Services of the New York Hospital. The criteria for use were the same as those described for the remaining cases.

which is consistent at all ages, of least degree in the 40–49 group and greatest degree in the 50–59.

TABLE 1.—AVERAGE SYSTOLIC AND DIASTOLIC PRESSURES, INCIDENCE OF HYPERTENSION AND AVERAGE WEIGHTS OF 900 PAROUS AND 900 NULLIPAROUS WOMEN ACCORDING TO AGE GROUPS.

Age group.	No.	Wt., kg.	Systolic pressure.			Diastolic pressure.			Incidence of hypertension.	
			Av.	S.D.	P.E.	Av.	S.D.	P.E.	No.	%
					Parous.					
20–29	200	59.1	117.8	10.0	± .48	73.4	9.3	± .45	2	1.0
30–39	200	64.8	125.1	11.4	± .55	77.9	8.9	± .42	8	4.0
40–49	200	67.0	135.5	15.4	± .74	82.7	9.5	± .45	36	18.0
50–59	200	67.5	148.0	20.6	± .99	86.8	11.0	± .53	56	28.0
60 or more	100	64.0	161.9	23.2	±1.55	89.6	11.8	± .79	51	51.0
					Nulliparous.					
20–29	200	55.3	119.6	9.5	± .46	73.2	7.1	± .34	4	2.0
30–39	200	58.6	124.3	13.4	± .64	75.7	9.1	± .44	11	5.5
40–49	200	62.7	133.5	20.4	± .98	81.9	11.2	± .54	37	18.5
50–59	200	66.2	144.4	24.6	±1.17	87.3	13.4	± .64	73	36.5
60 or more	100	60.9	158.0	30.8	±2.06	90.9	13.0	± .87	51	51.0

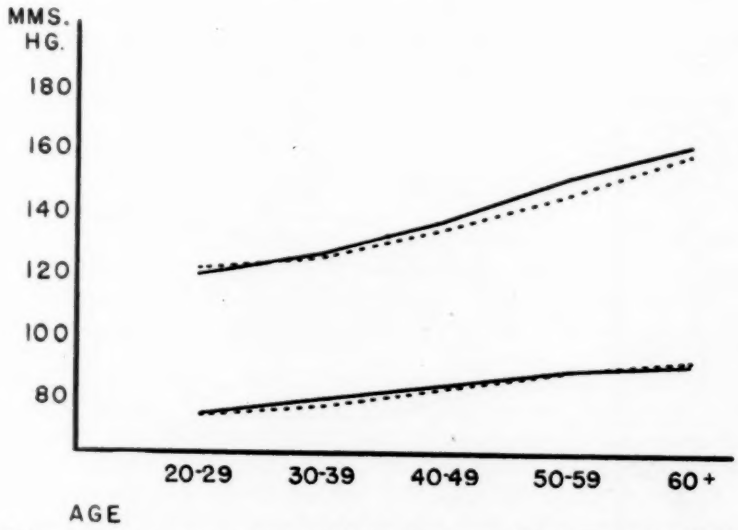

CHART 1.—The average systolic and diastolic pressures for parous (——) and nulliparous (– – –) women according to age groups.

In order to account for the factor of difference in body weight which might affect the systemic pressure,[5,12] the averaged weight of each age group is included in Table 1. It is noticeable that the parous woman has a definite and consistently higher body weight than the nulliparous patient (Chart 2).

In an attempt to determine whether repeated pregnancy produces evidence of vascular injury, the parous women were divided into

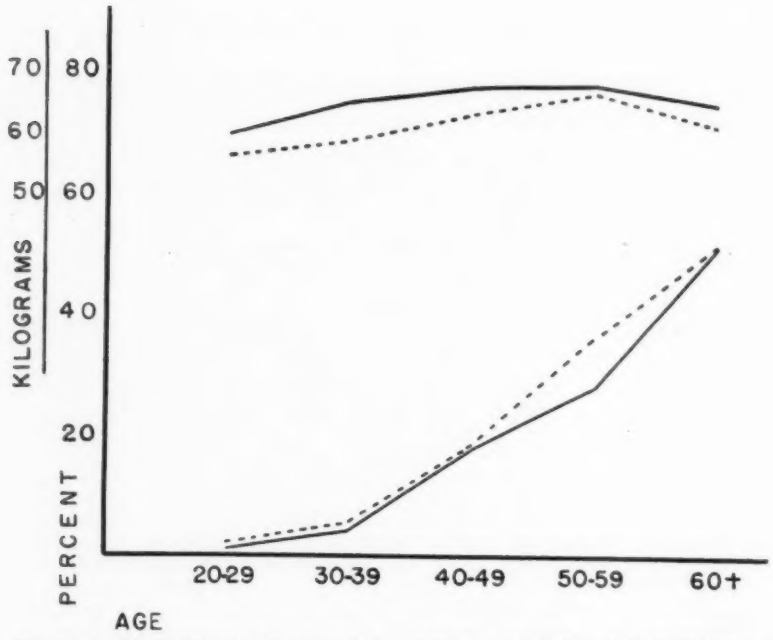

CHART 2.—The incidence of hypertension and the average body weight in kilograms of parous (——) and nulliparous (– – –) women according to age groups.

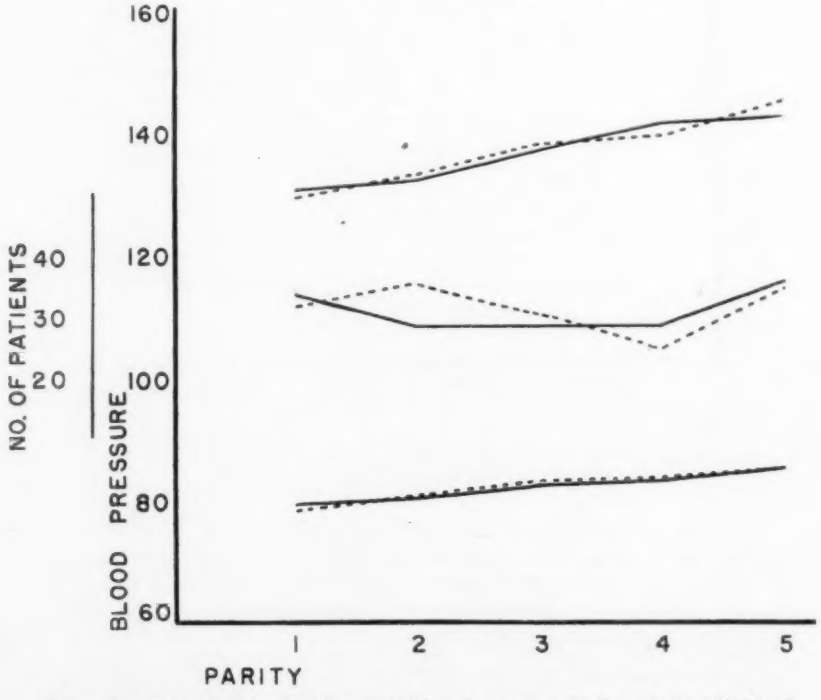

CHART 3.—The actual (——) and expected (– – –) average systolic and diastolic blood pressures and incidence of hypertension for women according to parity.

groups according to parity from 1 to 4 respectively, with all those having 5 or more parities being included in a fifth group.*

When the actual and theoretical average blood pressures for the parous patients are compared (Table 2), it is clearly shown that both the systolic and the diastolic pressures are closely parallel and that no significant differences can be made out (Chart 3).

The incidence of hypertension in patients of all parities (Table 2) also corresponds closely to the expected incidence for patients of those age groups, and no significant difference with ascending parity can be discovered (Chart 3).

TABLE 2.—THE ACTUAL AND EXPECTED AVERAGE SYSTOLIC AND DIASTOLIC PRESSURES AND INCIDENCE OF HYPERTENSION AMONG WOMEN OF DIFFERENT PARITIES.

		Actual average pressure.		Expected average pressure.		Incidence of hypertension.	
Parity.	No.	Systolic.	Diastolic.	Systolic.	Diastolic.	Actual.	Expected.
1	277	130.2	79.0	129.0	78.6	33	30.7
2	234	131.7	80.2	132.9	80.4	29	34.6
3	151	137.6	82.2	137.8	82.5	28	30.2
4	113	141.4	83.0	139.3	83.3	28	23.8
5 or more	125	142.6	85.3	145.2	85.3	35	34.3
Total	900					153	153.6

Because of a recent demonstration[6] that the average blood pressure fails to rise significantly with age following the removal from the series of patients who have hypertension, our age groups were recalculated after removal of all patients whose blood pressures averaged 140/90 mm. Hg or more.

It is shown in Table 3 that the systolic pressures following the removal of such hypertensive patients rise constantly in both groups, and that the average pressure for all age groups among the parous women exceeds slightly those levels seen among the nulliparous women.

The diastolic level rises, although more slowly than in the entire series, but fails to maintain its rising level in the parous woman. No more significant effect of pregnancy is noted here than before the removal of the hypertensive patients.

Discussion. The blood pressure levels and the incidence of hypertension in both parous and nulliparous women are well above those usually cited for the male,[5,12] and it seems reasonable to suppose that the vascular system of the female *per se* has an increased tendency toward this disease.

This work has been done on the assumption that the patients who have elevations of blood pressure are of sufficient number to cause a noticeable increase of the blood pressure level and of the

* The number of patients of each age group was determined for each parity. The average blood pressure of each parity group was then compared with that of a theoretical group composed of cases equally distributed with respect to age. The same procedure was carried out for the incidence of hypertension.

incidence of hypertension in random samples of parous women. To test this assumption it is necessary to determine the expected incidence of hypertension in all parous women if one assumes that some "toxemias of pregnancy" produce residual hypertension.

The "toxemias of late pregnancy" occur in approximately 10% of all women who approximate term.[9] Of these, 40% to 50% will develop one of the hypertensive states[2,4,10] (hypertensive disease or chronic nephritis). Therefore one would expect to find from 4 to 5 patients in every 100 parous women in addition to the average incidence for that age group.*

One would therefore expect to find at least 8 patients more with hypertension in each age group when compared with the nulliparous woman.

The average blood pressure level is not such an accurate index to use for determining any addition of small numbers of hypertensive patients to a large group of normal patients. When 4 patients with blood pressure elevations of 40 mm. Hg above the average are added in a group of 100 patients, the absolute rise in level for this group is only 1.5 mm. Hg. A rise of double this amount would be necessary to be of statistical significance (three times the probable error for this method).

We are unable to demonstrate any additional hypertensive patients for any of our age groups of parous women when compared with the nulliparous. This would seem to indicate that if the "toxemias" act as an etiologic agent in the production of hypertension, they do so to an extremely small degree, and that to all intents and purposes the patients having hypertension following "toxemia of pregnancy" are the same patients who would have hypertension had they not been pregnant. It also seems likely that the pregnancy neither aggravates this "latent hypertension" nor makes it obvious earlier, because one finds no increase in the incidence of hypertension in the earlier age groups when these are compared with the nulliparous groups.

TABLE 3.—AVERAGE SYSTOLIC AND DIASTOLIC PRESSURES IN VARYING AGE GROUPS FOLLOWING THE REMOVAL OF ALL "HYPERTENSIVE" PATIENTS IN BOTH PAROUS AND NULLIPAROUS WOMEN.

	Parous average.		Nulliparous average.	
Age group.	Systolic.	Diastolic.	Systolic.	Diastolic.
20–29	117.5	73.2	119.1	72.8
30–39	123.3	76.7	122.1	74.4
40–49	131.5	79.8	127.4	78.4
50–59	140.0	81.8	130.9	80.6
60 or more	146.5	80.6	141.3	81.8

The observation that the most rapid increase in both average blood pressure and the incidence of hypertension is noted following

* This would be obvious only in the group below 50 years of age, for cases above this level would for the most part be absorbed by the high "normal" incidence of the disease in these age groups.

the childbearing period, *i. e.*, at 40 years of age and above, coupled with a failure to find any significant difference in the parous woman when compared with the nullipara in the younger age groups would seem to rule out pregnancy and its complications as an important etiologic factor either in the causation or in the aggravation of this disease.

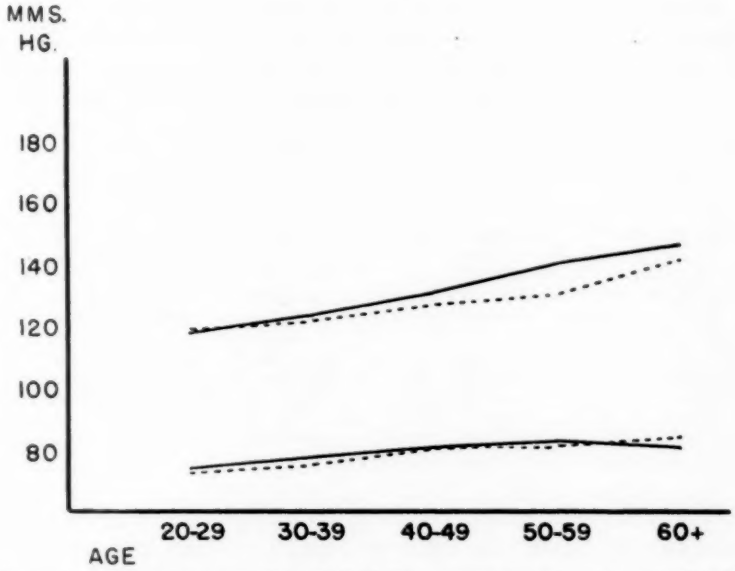

CHART 4.—The average systolic and diastolic pressures for parous (——) and nulliparous (– – –) women following the removal of all hypertensive cases.

It would seem likely that the apparent minimal increase in systolic level in the parous woman could be explained by the increased body weight which obtains in this group,[7,8] and the failure to find a similar difference, however slight, in the diastolic pressure would further tend to show that this elevation was not caused by vascular or renal injury as such.

The lack of effect of multiparity on the blood pressure and the incidence of hypertension is of interest. The women who have had repeated pregnancies have not only exposed their vascular systems to the "strain of pregnancy" several times but have also greatly increased their chances of having "toxemia of pregnancy." Despite these factors their blood pressure level and incidence of hypertension is no different from that of the nulliparous patient of the same age.

Following the removal of all hypertensive patients from the group, the average systolic blood pressure still shows a slightly higher range in the parous patient, while the diastolic remains essentially the same. This would tend to indicate that the hyper-

tensive patients present in each group were relatively equal in number and in the degree of hypertension experienced, and that the slightly higher level of systolic pressure among the parous women was not due to the inclusion of a number of "hypertensives."

Conclusions. No demonstrable difference can be noted in the incidence of hypertension and the average blood pressure levels of parous and nulliparous women.

It seems likely that the hypertension and hypertension-producing diseases which occur following a large portion of the "toxemias of pregnancy" are not the result of this complication of pregnancy, but rather that this complication of pregnancy occurs for the most part, if not exclusively, in patients whose vascular systems are endowed with the tendency toward hypertensive disease.

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PRIMARY THROMBOSIS OF THE AXILLARY AND SUBCLAVIAN VEIN.

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Primary thrombosis of the axillary and subclavian vein is apparently an uncommon condition. Matas[11] in his classic paper listed 100 cases that he had found in the literature, and since that time some other cases have been reported. The diagnosis of the condition is not difficult. Kaplan[8] has listed his criteria for diagnosis, all of which, however, need not be satisfied in a given case. There must, of course, be a swelling and cyanosis of the arm, and dilated superficial veins over the affected arm and anterior chest wall. An increased venous pressure, and prolongation of the circulation time on the affected side are corollaries to be expected from the nature

of the condition. However, these findings are not absolute because they tend to be normalized after the acute phase has disappeared and sufficient collateral circulation has developed. Many of the cases reported did not have the tender cord in the axilla, mentioned as a requisite by Kaplan. The presence of this finding depends on how much of the vein is involved in the thrombosis. Finally, Roentgen ray "visualization" with radio-opaque dyes, and infrared photography of the collateral veins are luxuries in the diagnosis of this condition. An innocuous substance like Diodrast should be used. Certainly there is no need to subject the patient to the injection of the thorium dioxide for "visualization," considering the potential dangers of this substance.

Many of the contributors to the literature of this disease have given their idea of its etiology. In nearly all cases there was a history of some form of sudden injury, often minor. In 2 of our cases there was no immediate antecedent trauma. There are several such cases reported in the literature. Nearly all the cases described were young men in good health. The right arm was more often involved, and when the left arm was involved it was usually in left-handed individuals.

There is a wide diversity of opinion as to the etiology of the condition. Schrötter,[14] who in 1884 first described this condition, thought that it was caused by phlebitis following sudden stretching and compression of the vein. Gould and Patey[6] found by anatomical study, that there exists a delicate subclavius-axillary valve in the vein where it underlies the subclavius muscle. When the subclavius muscle was suddenly stretched with the arm in abduction, the increase in venous pressure was supposed to have ruptured the valve and resulted in thrombosis. Cadenat[3] and Lahaussois[9] thought that respiratory effort also played a part by distending the axillary and subclavian veins and damaging the endothelium. Lowenstein,[10] working on cadavers, found that in certain of these, when the arm was in an abducted position, the costocoracoid ligament and subclavius muscle indented the axillary vein. This caused a rise in the venous pressure and a distention of the vein. Lowenstein also believed that forced expiration played a part in the slowing of the blood stream. Cottalorda[4] postulated that trauma produced an irritation of the perivenous sympathetic plexus, with resultant venospasm, and thrombosis. In Cottalorda's case, relief was obtained by excising a segment of the vein involved. In some of the cases described in the literature, thrombi were not found, which makes venospasm a possibility. Rosenthal and Lohr believed that in addition to the mechanical factors, there were also chemical changes in the blood caused by catabolites of abnormal metabolism. This would determine, theoretically at least, which persons would have thrombus formation, conceding a common mechanical basis. Veal,[16] and Veal and McFetridge[17] made a radical departure from

the recorded literature by claiming that on hyperabduction of the arm, the constriction of the vein occurred not over the first rib beneath the subclavius muscle, but below the head of the humerus and against the subscapularis muscle. They included two drawings which showed such a constriction of the subclavian vein at this point. They believed to have experimentally demonstrated that with the arm abducted, and under forced respirations, there were wide fluctuations in axillary venous pressure. This might lead to thrombosis by injury to the vein wall. They did not feel that the venous spasm of Cottalorda was much of a factor in producing the thrombosis.

The physical and laboratory findings in the recorded cases were not all similar. Cottalorda[4] found both the systolic and diastolic pressure to be higher on the affected side. One worker found no difference in these readings between the affected and unaffected side. Another described a decrease in the systolic pressure, while in the classical case of Matas the blood pressure was increased on the affected side. Horton[7] found a lowering of the oxygen capacity of the venous blood on the affected side (43 vol. % against 70 vol. %). Lohr found an increased red blood count on the affected side. Matas found a normal bleeding and coagulation time on his subject.

Case Reports. Case 1.—M. B., a 17-year-old adult male, in apparent good health, observed that in the year previous to admission his entire right upper extremity had progressively increased in width with a concomitant bluish discoloration. After a strenuous basketball game, he noticed a marked engorgement of the superficial veins of the shoulder and arm and felt a sensation of tension and a mass in the right axilla, when attempting to raise his arm. There was no pain or dysfunction. He had always been in good health. Four years prior to this event, while wrestling, his neck was twisted; the head was pulled over to the right for a day, after which he had no further trouble.

Examination revealed that the right pectoral and scapular regions, and right side of the neck appeared much fuller than the corresponding areas on the left. Superficial veins of the right upper extremity and pectoral area were markedly engorged, tortuous and numerous. The hand and arm had a cyanotic flush.

Measurements: right forearm, 28.2 cm.; left forearm, 27 cm.; right arm, 30 cm.; left arm, 28 cm.

Arterial pulse and blood pressure were equal on both sides. Circulation time on both sides was 18 seconds. The venous pressure in the right brachial vein was 34 cm., that of the left was 16 cm. Roentgen rays of the chest, arm and neck were negative.

Venography with Diodrast through the cephalic vein of the left arm revealed normal flow through the vein to the chest wall, which corresponded to the normal site of the subclavian vessel. From then on, all trace of the Diodrast in the vein was lost. On the right side (Fig. 1) the vein was equally well seen up to a point 2 inches externally to the chest wall. In addition, one could see numerous redundant worm-like coils indicative of collateral circulation of the deeper veins of the arm. Also, there was an abrupt blockage of the vein at the normal site of the subclavian, and at this point a collateral vessel into the vein of the neck could be demonstrated. This patient was subsequently seen again 5 years after the onset of the condition. Although the affected extremity was still as large as before,

and the collateral circulation over the surface of the arm and chest wall was still very prominent, the patient had no apparent difficulty in the ordinary use of his extremity.

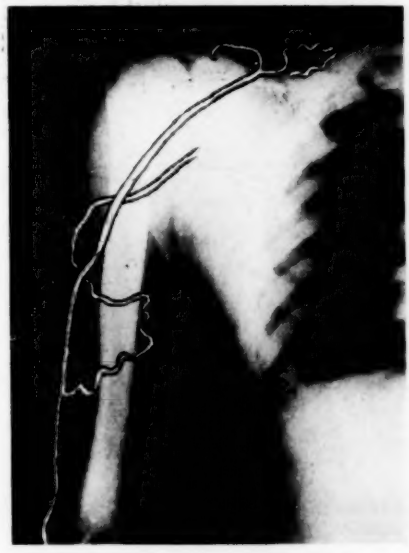

FIG. 1.—Venograph of Case 1, showing the abrupt termination of continuity of the axillary vein about 2 inches from the first rib margin. (Retouched.)

CASE 2.—Y. S., 20-year-old female adult, in good health, suddenly noted a swelling and redness of the right upper extremity 1 week before admission. There was no pain or dysfunction. She had always been in good health, except for a bout of rheumatic fever at 11 years of age which involved her knees and ankles for 2 or 3 months. She was left with no murmurs or other rheumatic stigmata. Three years before admission, she ran into a wall, spraining the right elbow, which was kept bandaged for a few days.

Examination revealed a very well-developed athletic young lady; all physical findings were normal, except for the right upper extremity which showed a non-pitting swelling from the pectoral region to the fingers, and was of a reddish-purple hue. There was no axillary adenopathy, nor was there a tender cord palpable in the axilla. The pulses were equal. The right superficial veins were prominent and tortuous and extended to the right anterior chest wall.

Measurements: right axillary region, 28 cm.; left axillary region, 25 cm.; right midarm, 24.5 cm.; left midarm, 22 cm.; right elbow, 23.5 cm.; left elbow, 22 cm.

The Roentgen rays of the chest, shoulder and arm were negative. Bronchoscopy and laryngoscopy were negative. The venous pressure taken at the cephalic vein on the right side was 24.5 cm.; on the left side, 12 cm. The circulation time by the calcium method on the right was 10.2 seconds, and on the left 8.8 seconds. A Roentgen ray study with Diodrast (Fig. 2), injected at the right cephalic vein, showed pronounced tortuosity of one of the vessels which passed up over the shoulder and into the cervical region.

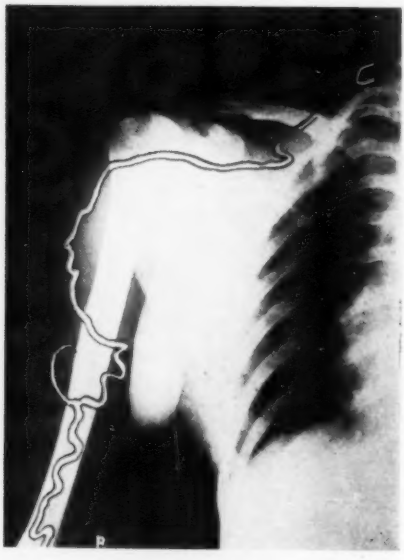

FIG. 2.—Venograph of Case 2. The axillary vein appears to be thrombosed throughout its entire extent and the collateral veins only are seen. (Retouched.)

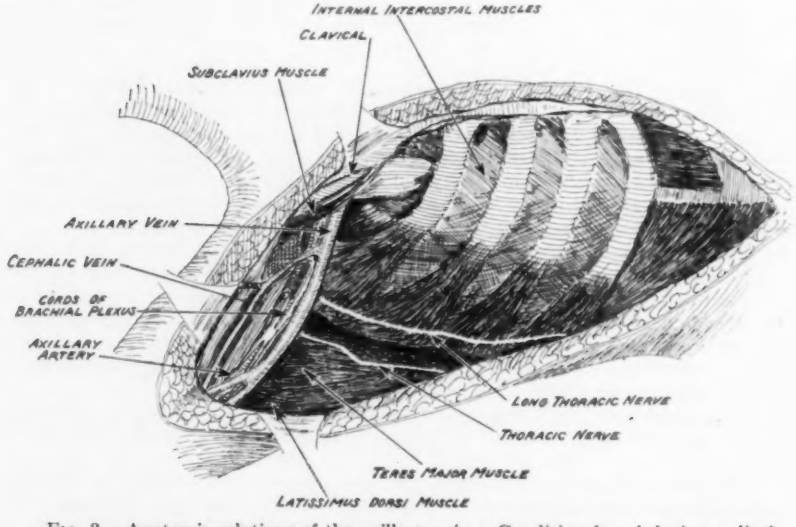

FIG. 3.—Anatomic relations of the axillary vein. Condition found during radical mastectomy operation with upper extremity of the patient abducted and externally rotated. There is stretching of the axillary vein but nearly uniform stretching. (Modified from Cutler and Zollinger, Atlas of Surgical Operations, courtesy of The Macmillan Company.)

It was believed that none of the dye had entered the axillary or subclavian vein. The oxygen content of the whole blood was 12.3 vol. % on the right side and 14.8 vol. % on the left side. She was last seen about 9 months after her first hospital stay and showed a slight decrease in the swelling and venous engorgement of the affected extremity.

CASE 3.—I. B., age 23, a well-developed male adult, struck his arm against a wall 3 days before admission. There was some immediate pain, followed by more pain and swelling the following day. For the ensuing 3 days the arm was enlarged and firm.

Examination was negative except for the right upper extremity which showed edema from the shoulder to the fingers, slightly pitting. The extremity was warm and had a mottled appearance.

Measurements: right axilla, 16.5 cm.; left axilla, 15 cm.; right midarm, 12.25 cm.; left midarm, 10.25 cm.; right mid-forearm, 10.5 cm.; left mid-forearm, 8.75 cm.

The Roentgen rays of shoulder and chest were negative. However, they did reveal abnormal, anteriorly placed scapulæ. A Roentgen ray "visualization" of this case was not done. Forty-eight hours after admission, the patient felt that his pain and swelling were relieved and he was discharged. He was not seen subsequently.

Comment. Venous thrombosis may be considered as the product of several factors, more than one of which must usually be present. These are as follows:[5]

1. Change in the vessel wall due to injury.
2. Change in the condition of the blood. This refers to a change in the blood composition and the formed elements of the blood.
3. Change in circulatory condition, such as slowing of the blood stream, the presence of an abnormally low blood pressure, and the formation of eddies in the veins.

1. *Changes in the vessel wall* may be due to injury of the vessel wall itself or to the valve in the vein. Histologically, the vein wall cannot be divided definitely into three coats as can the artery. The endothelium provides the smooth, internal lining to the vessels. The valves are reduplications of the endothelium, and have their free edges in the direction of the blood stream. The vein wall also contains collagen, elastic and smooth muscle fibers. The collagen fibers are somewhat extensible. There is a spiral arrangement of the collagen fibers when the vein has to undergo much functional change in length. Triepel[15] found that certain veins lengthen as much as 50% with body movement, without effect on the venous pressure, but that the resistance rose rapidly when the lengthening reached 55% of the initial size. Here then, we have one cause for the injury to the vein wall; overextensibility beyond the capacity of compensation of the collagen fibers. The elastic fibers are divided into two types; circular, to allow the vessel to accommodate itself to sudden increases in content and later restore normal caliber, and longitudinal to help prevent collapse in certain veins. Rancken[13] called attention to the fact that while the basilic vein at the elbow can lengthen from 20 to 27 cm., depending whether the arm is flexed or whether it is fully extended; these measurements only hold

true if the vein is not distended with blood. If it is, the vein will become tortuous on flexion. Since most veins are attached to the underlying structures, a sudden deformation of an attached tortuous vein may cause injury to its wall. Stimulation of peripheral nerves usually causes construction of the veins, *e. g.*, stimulation of the sciatic nerve causes constriction of the saphenous vein. Peripheral nerve injury might cause a prolonged spasm, and slow the venous return in the axillary and subclavian vein.

Valves are an important part of the vein structures. They are reduplications of the intima of the vein, and their free borders point in the direction of the blood stream. The most common position of valves in the venous trunks is just distal to the entrance of tributaries. Valves are found especially in those vessels that are subject to pressure from without, and those in the immediate sphere of muscular performance. The valve closes when the pressure in a segment of a vein proximal to it is greater than distal to it.

Gould and Patey[6] found a fairly constant valve in the axillary vein under the subclavius muscle, which they thought was delicate enough to be injured if the arm was forcedly raised. Speaking generally, a valve is a very difficult thing to injure, because of its situation and arrangement. The competence of valves and their integrity is guarded against by the pressure of the overlying structures. However, the possibility of the accident does exist.

Veal and McFetridge,[17] on the basis of a thorotrast Roentgen ray examination of one of their cases, came to the conclusion that hyperabduction and external rotation of the humerus causes a constriction of the axillary vein below the head of the humerus, and against the subscapularis muscle. In their study of fresh autopsy material they noticed that the vein was stretched proximal to the point of constriction. It was the good fortune of one of us (L. P.) personally to assist in two radical mastectomy operations and thus be able to test the theory of Veal and McFetridge in the living specimen. With the axillary vein exposed and on hyperabduction and external rotation of the humerus, the vein was seen to stretch, but the narrowing was not at one point, as indicated by these authors, but more uniformly distributed throughout the length of the vein (Fig. 3). This fact was checked many times in these patients. In both of these cases, there was apparently a subclavio-axillary valve present as demonstrated by lack of back flow when the axillary vein was stripped from this point.

In the case of Veal and McFetridge, the blockage of the vein occurred at a point between the situation of the head of the humerus and subscapularis muscle. In one of our cases in which contrast radiography of the venous system was done, and indeed in most of the recorded cases, the thrombus occurred in the subclavian vein at the inner border of the first rib. In another case that we have

described above, the thrombus had apparently extended throughout the entire axillary vessel so that it was not visualized at all in the contrast Roentgen ray. However, it is not for any one to say that a blockage at the point mentioned by Veal is impossible, but it certainly is not the only or usual point as is claimed by him.

Besides the factors enumerated above, mention must be made of several cases of severe venous spasm reported in Franklin's excellent monograph.[5] A severe venospasm, if it lasts for an appreciable length of time may so slow the venous circulation, as to be at least one factor in thrombosis of the vein.

2. *Change in the Condition of the Blood.* The following alterations in the blood, consequent on tissue injury are important, in the cause of venous thrombosis.[1]

(*a*) Increase in the number of platelets and their tendency to clump together.

(*b*) Rise in fibrinogen concentration and its effect on the sinking rate of cells.

(*c*) Anhydremia by increasing the viscosity of the blood. However, important as are these factors, local tissue injury of the vein is necessary to produce thrombosis. These changes in the blood are purely local at the site of thrombosis, as examination reveals normal bleeding and coagulation time and platelet count.

3. *Changes in the Circulatory Conditions.* The factors which influence the bloodflow in the veins of the body are: the *vis a tergo* from the left ventricle, the suction effect of respiratory movements, the muscular support and massage of the vein wall, the support by the abdominal muscles, and the effect of gravity above the cardiac level.

The most important factor in slowing the venous stream in the absence of cardiac failure, is some radical change in the normal variations in the sub-atmospheric pressure of the thorax. In the Valsalva experiment, which consists of a forced expiratory effort with the glottis closed at the end of deep inspiration, the tractive power of the elastic lungs is superseded temporarily because of the high pressure in the respiratory system. The intrathoracic venous pressure rises and the venous return is seriously hindered. According to Moritz and Tabora[12] and Bürger,[2] the venous pressure rises to 400 to 500 mm. of water during the Valsalva experiment. In everyday life, defecation, parturition, coitus and coughing constitute modified Valsalva experiments. The normal venous pressure varies between 40 and 110 mm. of water. In the excitement stage of anesthesia, there is also a considerable increase of venous pressure.

As regards the influence of exercise on venous pressure, Bürger differentiates two types: prolonged activities of speed and delicacy which have little effect on the venous pressure, and extreme efforts of short duration which have a marked effect. In the latter type,

which is demonstrated by weight lifting, high jump, diving, and swimming under water, there is closure of the glottis at the end of inspiration and contraction of the expiratory muscles. This results in the compression of the thoracic content and the heart. The venous return is markedly impeded and there is marked rise in venous pressure. This description duplicates the conditions of the Valsalva experiment.

In the first 2 cases that we have reported in this paper, it would appear at first glance that none of the three factors discussed, *i. e.*, vein injury, blood changes, or circulatory slowing, are operative. However, both had sustained accidents several years before, which probably left them with some slight injury of the vein wall. At some fortuitous moment of circulatory slowing (any of the everyday duplications of the Valsalva experiment) this combination of injury and stasis could result in thrombosis. The third case had a definite pertinent injury, preceding the thrombosis, as seems to be true of most of the cases in the recorded literature. While this condition is called either "primary" or "effort" thrombosis in the literature, the appearance of some definite cases without immediate antecedent injury has tempted certain authors to label this condition as "so-called" effort thrombosis of the axillary and subclavian veins. We are of the opinion that an injury more or less violent probably antedates most of the cases, but this injury need not immediately precede the thrombosis.

Summary. 1. The literature concerning primary or effort thrombosis of the axillary and subclavian veins has been reviewed.

2. Three personally observed cases, 2 of which were accompanied by venography, were described.

3. The possible causes of this condition were evaluated.

4. Two of our patients could *not* recall any injury immediately preceding the onset of the condition, but did give a history of injury sometime before the thrombosis.

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THE EFFECT OF A BARBITURIC ACID DERIVATIVE ON THE LOBELINE CIRCULATION TIME.

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In a previous paper,[1] one of us (K. B.) reported his experience with alpha lobeline hydrochloride as an agent for measuring the velocity of bloodflow, a method originally proposed by Teplov and Sor.[18] Lobeline stimulates the respiratory center, probably by way of a reflex from the carotid sinus, and thus produces cough. The reliability of the test, therefore, would seem to depend on the normal functioning of the respiratory center. In clinical medicine, a number of drugs are frequently employed which are known to depress the respiratory center. It occurred to us that the administration of such drugs might influence the results of the lobeline test. The present study was undertaken in order to determine the existence of such an influence. As a drug suitable for this investigation, a rapidly acting barbiturate, sodium propyl-methyl-carbinyl allyl barbiturate (Seconal*)[17] was chosen.

Method. Fifty patients were used in this study; 38 males and 12 females. The patients varied in age from 14 to 75 years (average age 33). Seven were patients suffering from heart disease; 5 of these were in congestive heart failure. The remaining 43 patients suffered from various other conditions such as are commonly encountered in a general medical ward. All patients were in bed. None had fever. The tests were made in the morning between breakfast and lunch.

The technique of the test has been previously described in detail. Only a brief description need be given here: 3 to 5 mg. of alpha lobeline hydrochloride (0.3 to 0.5 cc. of the 1% solution†) are rapidly injected into an antecubital vein. Several seconds later, the patient coughs. The time interval from the beginning of the injection to the onset of coughing represents the "circulation time" for that patient. If cough fails to appear, the test is repeated with 7.5 to 10 mg. of the drug, 10 to 15 minutes later.

In the present study, Seconal was administered by mouth immediately after the successful completion of the test. Thirty patients received 0.18 gm., while 20 received 0.27 gm. After an interval varying from 30 to 90 minutes (usually 60 minutes) the degree of hypnotic effect of Seconal was noted and the lobeline test was repeated. It was found that the hypnotic effect of 0.27 gm. of Seconal was frequently not greater than that of 0.18 gm. This is not surprising since a number of other factors (age, weight, speed of absorption, and others) were operative. Therefore, the patients were divided into three groups according to the degree of hypnotic effect produced, and not according to the dose of Seconal given. Group 1

* Manufactured by Eli Lilly and Company.

† The ampules of alpha lobeline hydrochloride used in this study were supplied by the Sandoz Company

included those patients who were found to be awake at the time of the second test. Group 2 included those patients who were found asleep at the time of the second test, but who awakened at the touch of the examiner. Group 3 comprised those patients who remained asleep until insertion of the needle. A few patients in this group actually slept throughout the entire procedure.

In each case, the results of the first and second lobeline test were compared and the difference was expressed in terms of percentage of the first result.

In addition, duplicate tests were made on 100 patients without administering Seconal. The time interval between these duplicate tests varied from 15 to 60 minutes. These tests, which are reported in detail in a separate paper,[9] served as a control series.

Results. The difference in lobeline circulation time before and after the administration of Seconal was striking in most instances. Table 1 shows the results obtained in each of the three groups described above.

TABLE 1.—RESULTS OF LOBELINE CIRCULATION TIME TEST BEFORE AND AFTER THE ADMINISTRATION OF SECONAL—50 CASES.

Group 1 (minimal hypnotic effect).				Group 2 (light sleep).				Group 3 (deep sleep).			
Case No.	First test (sec.).	Second test (sec.).	% difference.	Case No.	First test (sec.).	Second test (sec.).	% difference.	Case No.	First test (sec.).	Second test (sec.).	% difference.
1	5.1	7.5	+ 47	1	11.1	14.3	+ 29	1	16.4	66.0	+302
2	7.5	8.2	+ 9	2	10.1	13.0	+ 29	2	16.0	20.3	+ 27
3	13.6	10.0	− 27	3	11.2	10.6	− 5	3	10.4	70.0	+573
4	9.5	5.2	− 45	4	8.1	8.4	+ 4	4	15.0	40.0	+167
5	8.0	4.8	− 40	5	4.1	5.6	+ 37	5	11.0	12.9	+ 17
6	17.4	17.6	+ 1	6	10.0	12.3	+ 23	6	8.2	52.4	+539
7	10.0	8.1	− 19	7	4.4	5.4	+ 23	7	12.4	17.2	+ 39
8	15.9	10.3	− 35	8	7.9	10.1	+ 29	8	8.4	12.0	+ 43
9	8.1	8.0	− 1	9	15.9	16.5	+ 4	9	3.0	9.0	+200
10	7.2	4.9	− 32	10	7.5	8.2	+ 9	10	12.1	16.7	+ 38
11	8.2	21.2	+159	11	5.6	10.8	+ 93	11	8.9	10.9	+ 22
12	17.7	18.5	+ 5	12	7.4	9.5	+ 28	12	8.2	failure	—
				13	7.2	24.1	+235	13	5.0	18.4	+268
				14	7.6	10.2	+ 34	14	27.9	12.8	− 54
				15	20.5	failure	—	15	9.2	19.9	+116
				16	8.8	25.2	+186	16	6.0	12.6	+110
				17	4.9	6.0	+ 22	17	45.0	23.0	− 49
				18	12.8	44.0	+244	18	11.3	failure	—
				19	17.2	24.8	+ 44				
				20	6.6	9.7	+ 47				

Only in Group 1 (minimal hypnotic effect) were the differences between the first and second lobeline circulation time relatively minor, and only in this group was the second circulation time shortened more frequently than it was prolonged (shortened in 7 cases by an average of 28%, prolonged in 5 cases by an average of 44%).

In Groups 2 and 3, where actual sleep was induced, considerable differences between the results of the first and second test were found in most instances, and there usually was marked prolongation of the second lobeline circulation time. In Group 2 (light sleep), 18 out of 20 cases showed prolongation after Seconal, and the average degree of prolongation was 62%. In this group, the second circulation time was shorter in only 1 case (by 5%), and in 1 case, the

second test resulted in failure. In Group 3 (deep sleep), 14 out of 18 cases showed prolongation of the lobeline circulation time after Seconal, and the average degree of prolongation was greatest (176%). In this group, the lobeline circulation time was shortened after Seconal in 2 cases (by 54% and 49% respectively); in 2 additional cases, the second test resulted in failure.

In the control series of duplicate measurements, where no Seconal was administered, the average difference between the first and second test was 31%. The result of the second test, furthermore, was higher about as frequently as it was lower (higher in 44 cases, lower in 41 cases, identical in 3 cases). Besides, the differences in results were for the most part minor. The greatest single difference in results of the control series was 199%, while after Seconal, prolongations of as much as 573% occurred (in Group 3).

TABLE 2.—COMPARISON OF SECONAL SERIES WITH CONTROL SERIES.

				Results of second test higher.		Results of second test lower.		
	No. of cases.	Results of both tests identical.	Second test a failure.	No. of cases.	Average difference between first and second test.	No. of cases.	Average difference between first and second test.	All cases, average difference between first and second test.
Control Series (no Seconal given)	100	3	12	44	37%	41	19%	31%
Seconal Series:								
Group 1 (min. hypnotic effect)	12	0	0	5	44%	7	28%	35%
Group 2 (light sleep)	20	0	1	18	62%	1	5%	59%
Group 3 (deep sleep)	18	0	2	14	176%	2	52%	160%

Comment. The contrast between the Seconal series and the control series (Table 2) furnishes striking evidence of the effect of Seconal. If we compare the results obtained in each of the three groups of the Seconal series (Table 2), we find additional evidence of seconal effect. In Group 1 (minimal hypnotic effect), the average difference between the first and second test is not great and shortening of lobeline circulation time occurs about as frequently as prolongation. With more marked hypnotic effect, however, the average difference between the first and second test rises progressively, and shortening of the second lobeline circulation time becomes exceptional.

Our results, therefore, lead us to conclude that the administration of a barbiturate such as Seconal prolongs lobeline circulation time.

An explanation of this prolongation is found if we consider the mode of action of each drug. The first, lobeline, is known to stimulate the respiratory center,[16] probably by way of a reflex from the carotid sinus.[7] One of the manifestations of such stimulation is cough. The second drug, Seconal, belongs to that group of hypnotics which depress the centers in the brain stem, particularly those

of the thalamic and subthalamic regions by which the state of sleep is induced. Vital centers in the medulla such as the respiratory, cardiac, and vasomotor centers are simultaneously depressed.[11] Pharmacologic experiments on cats show that the respiratory center is the one primarily and predominantly affected.[3,4] When a single sublethal dose of one of the barbiturates (sodium ethyl [1-methyl butyl] thiobarbiturate*) is employed, the first undesirable effect is sudden cessation of respiration; only after repeated doses are cardiovascular effects in evidence.[13] The heart action does not stop unless respiration ceases. As a rule, death is due to respiratory failure.[5,12] While there may be differences in action between the individual members of the barbiturate group, they all, including Seconal, depress the respiratory center. Thus, they counteract the effect of lobeline, and it is undoubtedly this counteraction which results in the delayed cough response to alpha lobeline. In addition, there is experimental evidence to show that drugs of the barbiturate group depress the carotid sinus.[2,10,19] This would also interfere with the effect of lobeline.

At this point, the question may be raised whether the prolongation of lobeline circulation time found by us is really caused by depression of the respiratory center, or whether it is caused by actual slowing of the velocity of bloodflow. Experimental evidence is available to answer this question. Schröder and Böckmann[15] recently investigated the effect of two barbiturates on the velocity of bloodflow. These authors produced deep anesthesia by intravenous injection of Eunarcon (sodium bromallyl-isopropyl-N-methyl-barbiturate) in 5 patients, and by intravenous injection of Evipan (methyl-cyclo-hexenyl-N-methyl barbituric acid) in 5 additional patients. The circulation time was measured by Koch's fluorescine method[8] before, during, and 6 hours after anesthesia. Prolongation of circulation time was never found; shortening was found occasionally.

It is fair to assume, therefore, that the prolongation of lobeline circulation time found by us does not represent an actual slowing of bloodflow, but rather a prolongation of reaction time. This interpretation is borne out by the not infrequent occurrence of inordinate prolongation (up to 573%) of lobeline circulation time in Group 3 of our series, since such extreme slowing of bloodflow could hardly occur.

From our observations, certain conclusions of practical significance may be drawn. Measurements of circulation time by means of lobeline should not be made on patients who are under the influence of barbiturates or other drugs (*e. g.*, morphine) which depress the respiratory center. Since such drugs are used very frequently, this constitutes a definite limitation of the usefulness of the test. Similarly, the lobeline test should not be employed in the presence

* Sodium pentothal.

of pathologic states which are associated with Cheyne-Stokes breathing or other forms of depression of the respiratory center. This second limitation is particularly regrettable, for it had been hoped that the lobeline test, because of its objective end point, would be useful for the measurement of circulation time in conditions such as cerebral accident, uremia, diabetic coma, etc.

Moreover, what applies to lobeline also applies to any other method of measuring velocity of bloodflow which depends for its result on the respiratory center or the carotid sinus. Such methods include the sodium cyanide test[14] and the carbon dioxide inhalation method.[6] None of these tests should be employed when there is reason to believe that the respiratory center is depressed either by drugs or by pathologic states.

At this point, another comment may be in order. Barbiturates are widely used, and it is commonly assumed that ordinary hypnotic doses have no appreciable effect on the respiratory center. The results of our study, in which such doses were used, indicate that the depressant effect on the respiratory center is greater than is generally appreciated. In this connection, Cheyne-Stokes breathing should again be mentioned. Patients exhibiting this type of breathing are usually restless and sleep poorly. They, therefore, frequently receive barbiturates in considerable amounts. Our observations indicate that this medication may intensify the respiraratory disturbance. It would appear that caution in the use of barbiturates is advisable in such cases.

Summary. 1. The lobeline circulation time test was performed in 50 patients, before and after the administration of Seconal.

2. In 12 patients, hypnotic effect of Seconal was minimal. In these, shortening of the lobeline circulation time occurred more frequently than prolongation, and the difference between the first and second test were, for the most part, minor.

3. In 20 patients, light sleep was produced. In 18 of these, the second test showed the lobeline circulation time to be prolonged by an average of 62%.

4. In 18 patients, deep sleep was produced. In 14 of these, the second test showed the lobeline circulation time to be prolonged by an average of 176%.

5. In a control series of 100 duplicate measurements of lobeline circulation time without administration of Seconal, shortening was found as frequently as prolongation, and the average difference between the results was only 31%.

Conclusions. 1. Seconal, a derivative of barbituric acid, prolongs the lobeline circulation time.

2. This prolongation is due to the depressant effect of Seconal on the respiratory center and the carotid sinus. It does not, therefore, represent a true slowing of bloodflow but rather an increase in reaction time.

3. The lobeline test or any other method for measuring circulation time which depends on the respiratory center or the carotid sinus for its result (sodium cyanide test, carbon dioxide inhalation method) should not be employed on patients whose respiratory center is depressed either by drugs (barbiturates, morphine) or by pathologic conditions, especially when Cheyne-Stokes breathing is present.

4. Ordinary hypnotic doses of a barbiturate may depress the respiratory center to a greater extent than is generally appreciated.

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ELECTRIC CONVULSION THERAPY IN PSYCHOSES.

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Convulsions artificially produced by drugs such as camphor, metrazol (cardiazol), triazol, ammonium chloride, nitrogen, and picrotoxin have now been used in treating psychoses for some 6 years.[1,3,5,7,11,14,15] During the last 3 years such treatment has been carried out on a large scale in the United States. The chief drug used has been metrazol. Its advantages and disadvantages, as well as dangers, are well known as are also the frequent disappointments and the occasional brilliant results following its use. At this time it can be definitely stated that convulsive therapy has achieved for itself a definite niche in the therapeutic armamentarium of those physicians concerned with mental disease.

In 1937 two Italian investigators, Cerletti and Bini[4,5,6] described

the use of electric current to produce seizures in dogs. After preliminary study of the technique of the use of the current as well as of the pathologic effects on the brains of animals these workers produced seizures in patients with schizophrenia. As pointed out by Furst,[10] there was some previous work on the use of electric current to provoke unconsciousness and seizures, but the Italian investigators deserve credit for initiating this technique in mental disease. The work was very soon taken up by English, German and American workers.[9,10,12,13,16,17]

Apparatus and Technique. The technical structure of the apparatus will not be discussed in detail. The earlier apparatus was so arranged that a testing current, which was a fractional part of the treatment current, was passed through the head of the patient to measure the electrical resistance of the patient's tissues. Following this the treatment current was calculated and administered for an accurately measured interval. The current used was 60 cycle A.C. suitably modified by transformers, etc. The newer apparatus, such as that used by the authors, consists of interlocked testing and treatment circuits. The testing current used is 1000 cycle A.C. current of approximately 0.1 milliampere which is imperceptible to the patient, not polarized, and accurately measures the resistance of tissues between the connected electrodes. Setting of the testing circuit automatically sets the treatment circuit to the desired intensity. An automatic electronic timer, variable from 0.05 to 1.0 second controls the duration of the delivered treatment current. The current used in treatment is 60 cycle A.C. as in the older apparatus. The machine includes safety devices which prevent the passage of too high a current or stray current through the patient and which prevent the passage of treatment current before testing current through the patient.

In administering the treatment the patient is made to lie down in bed, a pillow is placed under the dorsal region of the spine to hyperextend to some extent the vertebral column. Electrodes are applied fairly high in both temporal regions. It is not necessary to shave the hair from this portion of the head. Electrode paste such as is used in electrocardiography and electroencephalography is applied to the areas under the electrodes, or a gauze sponge saturated with 10% salt solution is used for electrical contact between the electrode and the scalp. The apparatus is set for the desired time interval, usually 0.2 to 0.5 second. The testing current is applied and adjusted to a level usually between 400 and 600 milliamperes and then the treatment current is applied. The patient's hands and arms are held flexed and adducted over the chest to prevent dislocation and injury to the shoulders. The thighs and legs are mildly restrained and, if possible, a soft cotton and gauze mouth gag is inserted into the mouth during the ensuing convulsion. The management of the convulsion itself is, therefore, exactly the same as the management of a metrazol seizure. The level of the current and the time interval in which it is applied must be experimentally determined at the time of the first treatment. We now routinely use 450 M.A. for 0.3 second as an initial dose because almost all patients will have seizures with this dose. The seizure itself is notable for the brevity or complete absence of the latent period in most instances. Occasionally, however, a latent period of as much as 30 to 40 seconds between application of current and convulsive seizure has been observed. Failure to produce a convulsion was formerly a frequent annoyance, but the newer apparatus seems to be without such fault. The earlier apparatus made to deliver no more than 500 M.A. was rightfully but unnecessarily too conservative. The newer apparatus delivers up to 700 M.A. which is apparently adequate

in the most electrically resistant patients. Occasionally application of the current fails to produce a major seizure, and results only in brief "absence" or "petit mal" type of automatic activity for some seconds. In such instances it is our practice to repeat the current after one minute at a higher level or for a longer period to produce a seizure. The duration of the seizure varies from 30 to 90 seconds and is usually shorter than the average metrazol seizure. The treatments have been given once or twice weekly in the group treated to date. Other types of apparatus were used in the earlier period of this work. Some used constant amounts of current with only time variable, this up to several seconds.

Curare (Bennett) has been used successfully to reduce the force of muscle contractions with electric shock in a number of patients who previously had suffered bone injuries during metrazol treatment in our own or other hands.

Our approach to this work was extremely conservative both in number of patients and in the frequency of seizures produced for the first 8 months. After this preliminary period, we have practically substituted the use of electric shock for metrazol in convulsive therapy. The number of patients in our series is, therefore, not so large as might be expected from our early association with this work.

The ease of producing a convulsion is to a great extent determined by the resistance of the patient's tissues to the passage of current. Certain factors have been found to have a definite influence in this respect. In extremely hot weather when the patient's skin is moist and the conductivity of the skin as a whole is greatly increased it is much harder to produce a seizure. This is apparently the result of the by-passing of a large portion of the current through the skin of the body so that relatively less and definitely insufficient current passes through the head to affect the brain. Under such conditions it is probably wise to postpone for some days any attempt to use this treatment. The use of air-conditioned chambers where they are available may be an answer to this difficulty. In colder weather where the skin resistance of the patient is increased it is sometimes (rarely, however) difficult to pass sufficient current through the head at all to produce a seizure. Under such circumstances the difficulty may be overcome by rubbing the electrode jelly more vigorously into the scalp or using more liberal libations of salt solution in the sponge between the electrode and the scalp. The intensity of the current can be raised somewhat and the duration of the application increased. The electrically produced convulsion differs slightly from that produced by metrazol. There is usually no latent period between application of the current and tonic phase of the epileptiform seizure. Only rarely is there a lapse of 5 to 10 seconds between current application and seizure. In a few instances latent periods of 20 to 40 seconds were observed. The convulsive movements are perhaps less violent in a certain proportion of cases than with metrazol, but this is certainly not always the case. The duration of the convulsive period between onset of

the tonic phase and the first long post-convulsive breath, is definitely less in the electric than in the metrazol convulsion if averages of a series of cases are considered. Convulsive periods as long as 93 seconds have been observed, however. Patients awaken more rapidly and with less confusion as a rule after electric than after metrazol seizures. There is complete amnesia for the period of the treatment and a short period before and after. Our most intelligent patients state that practically no sensation accompanies the treatment and they willingly return for subsequent treatments. It is only after several treatments that patients usually realize that placing the electrodes initiates a series of events for which they have no memory but the awakening.

TABLE 1.—RESULTS OF ELECTRIC SHOCK TREATMENT OF PSYCHOSES.

Duration, yrs.	Complete recovery.	Social remission.	Improved.	Unimproved.
Schizophrenia.				
0–1	1	2	9	1
1–2	0	3	2	1
2–5	1	2	8	5
Over 5	1	1	8	4
Total	3	8	27	11
Manic Depressive and Involutional.				
0–1	3	1	1	0
1–2	0	0	1	0
2–5	0	0	1	1
Over 5	2	0	2	1
Total	5	1	5	2
Other Diagnoses.				
0–1	1	0	1	0
1–2	0	2	0	0
2–5	0	0	0	0
Over 5	0	2	0	0
Total	1	4	1	0
Total all diagnoses	9	13	33	13

Total patients who completed treatment, 68

Complications: Dislocation of the jaw has been observed, but this is considered inconsequential because mandibles usually replace themselves or are easily pushed back into place. One fracture of the head of the humerus has been produced. No other major fractures of vertebral or long bones have occurred with electric seizures in our series even though numerous Roentgen ray examinations have been made. The lower alveolus with the four incisor teeth was found fractured after a seizure in one patient but since this patient was violent and self destructive at all times and since no reasonable mechanical relationship between the seizure and the fracture could be adduced this fracture is not considered due to the electric shock. No dislocation of the shoulders has been seen.

The most outstanding feature of the treatment is the total absence of fear of the treatment on the part of the patients who have had one or more treatments. Most of the patients repeatedly and willingly come to the clinic for the treatment and lie down voluntarily on the bed and allow the electrodes to be applied. This statement, of course, does not apply to patients who are negativistic and resistive to any procedure whatever, whether painless or not. After a series of treatments there seems to be less confusion developed than after a similar number of metrazol seizures. In only one instance did secondary seizures occur one or more hours after the treatment. This was in a woman with history of seizures in childhood. Effects on the heart mechanisms seem to be no different than those experienced from metrazol treatment.[2]

As might be expected, the results from electric convulsion therapy are hardly different from those which have been observed following metrazol. Early in our work with this treatment we used only patients who had been ill for long periods and whose prognosis was hopeless. Our figures, therefore, do not reflect the best that the treatment offers. The figures of our results (Table 1) and our clinical impressions from experience with this treatment lead to the following somewhat dogmatic assertions:

1. Convulsion therapy with electric shock (as with metrazol) is most effective in manic depressive psychoses of either manic or depressive preponderance, involution psychoses, and in some catatonic states.

2. Convulsion therapy only occasionally produces lasting benefit in hebephrenic or paranoid schizophrenic patients.

3. The results of treatment are favorable in inverse proportion to the duration of illness. Duration over one year of any psychosis is likely to be unfavorable.

4. In our experience no unfavorable effects on the psychoses have been produced by the treatment but progress of deterioration in old patients has been unaffected. No clinical evidence of irreversible neurologic damage has been encountered.

We have not thus far seen any patients whom we think have been made worse by the treatment or who have suffered any serious or permanent injury therefrom. No late complications have as yet been observed but we have been giving the treatment for only one year (April, 1941), and have treated 102 different patients with the production of 569 seizures.

Comparison between the electrical convulsions and those produced by metrazol from a therapeutic point of view is of some interest. The outstanding difference between the two methods of treatment is, of course, the relative absence of fear on the part of the patient of the electric seizure. This is, no doubt, due to the almost instantaneous effect of the current and to the complete amnesia of the patient for any part of the treatment. There is some retrograde

amnesia so that the patient does not usually remember even lying down and having the electrodes applied, and the awakening from the electric convulsions is more rapid and quiet as has been mentioned. The therapeutic results of the two methods seem to be practically parallel as far as expectations in the group treated can be considered. Electricity can produce seizures without the difficulty or danger attending intravenous injections. This is to be considered a distinct advantage.

Conclusions. We have completed electric shock treatment on 68 patients suffering from various psychoses. Nine were regarded as recovered; 13 had a "social remission;" 33 more were regarded as improved, and 13 as unimproved.

This interesting therapeutic method must still be considered experimental and not be generally applied without great caution and circumspection. Patients submitted to the treatment should be observed for a long period afterward to determine the possibility of late complications. If these should be no worse than the effects of metrazol, electric convulsion therapy must be considered a distinct adjunct to our present methods.

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THE ASSIMILATION RATE OF INTRAVENOUSLY INJECTED GLUCOSE IN HOSPITAL PATIENTS.

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In 1917, Sansum and Woodyatt,[1] and Wilder and Sansum,[2] found that the intravenous glucose tolerance limit in man and experimental animals was approximately 0.85 gm. per kilo per hour. The sub-

jects studied were normal, injections were continued over long periods of time, and the rate of injection was carefully controlled by a special pump. An 18% solution of glucose was used in most experiments, but it was found that wide variation in concentration did not influence the tolerance limit. When the rate of infusion exceeded 0.85 gm. per kilo per hour, glycosuria appeared promptly. With the higher injection rates, although glycosuria was constant, the amount of glucose lost in the urine was only a fraction of that injected, and by no means the total amount in excess of 0.85 gm. Glycosuria was observed to stop within a few minutes after discontinuing the injection. Under conclusions (1) they state: "For any individual case the rate at which glucose enters the blood determines the rate of glucose utilization and excretion . . . ." Very probably others have conducted similar studies since these reports were published, but we have been unable to find such references by a search of titles.

The object of the present study was to observe the intravenous glucose assimilation rate in hospital patients, and to learn to what extent this should be taken into account in the practical administration of glucose by vein for the maintenance of fluid balance and other purposes.

Method. The subjects studied were adult patients on the surgical service of Dr. E. T. Crossan. Weights were determined by estimate, or by questioning. Glucose was administered by an ordinary gravity apparatus, the rate of injection being calculated from the total amount given and the time consumed in the process, although every effort was made to keep the flow constant. Urine was collected for a period of 12 hours from the beginning of injection, and the amount of glucose excreted was determined by the method of Folin and Wu as for blood sugar, after treatment with Lloyd's reagent.

The more important data are recorded in Chart 1. Case 22 was found, on autopsy, to have a carcinoma of the pancreas and is thought to have been diabetic, although he did not have glycosuria or fasting hyperglycemia. His data were not included in the calculations. Observations are arranged in order of increasing injection rates of glucose per kilo per hour. Grams of glucose retained per kilo per hour are calculated by difference from grams given and grams lost. The chart shows grams of glucose given per kilo per hour plotted against grams retained per kilo per hour.

Discussion. The coefficient of correlation for grams of glucose given and grams retained is very high, 0.9898. This is in agreement with the statement of Sansum and Woodyatt quoted above, that the rate of injection determines the rate of utilization. The maximum utilization rate when a considerable quantity of glucose was given (Case 21) was 5.77 gm. per kilo per hour, or 87 gm. in 20 minutes. The assimilation of 18 gm. per kilo per hour when 25 gm. were injected in 1 minute (Case 22) is regarded too acute an experiment to be typical.

Of equal importance for the purpose of this study is the observation that there was essentially no correlation between the rate at which glucose was injected and the amount lost in the urine. The coefficient of correlation was 0.1063. All cases lost some glucose. This is not in agreement with the observations of Sansum and Woodyatt who found the rate of loss to be determined by the rate of injection, and who observed no glycosuria with low injection rates. That there should be glycosuria in all cases seems understandable, however. The injection of 100 cc. of 5% glucose would, by calculation, raise the level in 5000 cc. of blood from 100 to 200 mg. per 100 cc., at which concentration some glucose must reach the kidneys

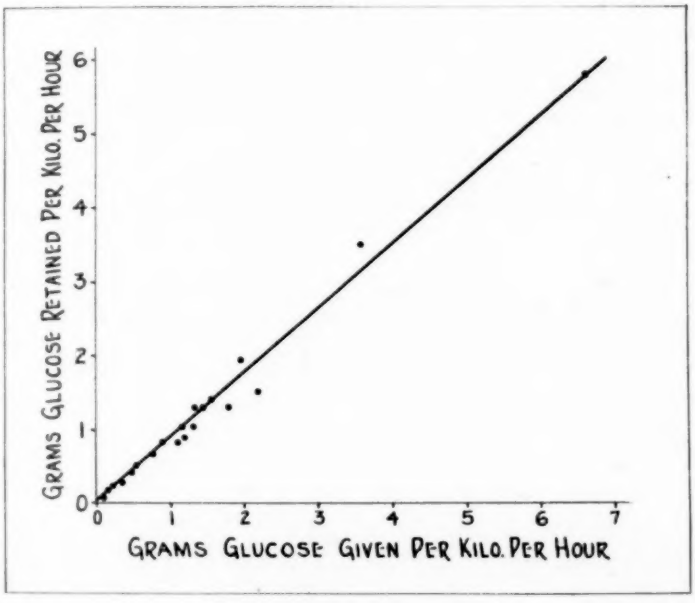

CHART 1.

before passage through liver and muscles. The occurrence of both small and large losses of glucose with both low and high rates of injection is interesting. The "spill" varied approximately from 0.3% to 30%, the average being 12.2%. For this we could find no explanation. The fasting blood sugar, the total amount of glucose given and the presence of fever were considered and found to be unrelated. Previous diet possibly had some effect, as glycosuria was slightly heavier in those who had fasted. This, however, was an inadequate explanation. From general knowledge of the many factors influencing carbohydrate tolerance, one might predict a wide variance in ill individuals in their ability to absorb glucose. In

addition to fever and previous diet, nervousness, temporary impairment of liver, pancreas, adrenals, thyroid and pituitary; the effect of such drugs as morphine, chloral, nembutal; acidosis; alkalosis; and inherited peculiarities, must effect sugar metabolism of the average surgical patient in varying degrees and combinations.

TABLE 1.—OBSERVATIONS ON THE INTRAVENOUS ADMINISTRATION OF GLUCOSE AT VARYING RATES TO HOSPITAL PATIENTS.

Case.	Diagnosis.	Weight (kilos).	Fasting blood sugar.	Fluid given.	Glucose given (gm.).	Glucose given (gm.), kilo/hr.	Glucose retained (gm.), kilo/hr.	Glucose lost, %.
1	Salpingitis	59	..	900 5%	45	0.073	0.072	0.533
2	Rupt. appendix	61.8	95	3500 5%	175	0.159	0.154	3.24
3	Rupt. gast. ulcer	61	107	4000 5%	200	0.222	0.217	1.5
4	Ac. appendix	80	90	2000 5%	100	0.390	0.290	27.20
5	Rupt. appendix	36	110	1000 5%	50	0.463	0.458	0.944
6	Uterine fibroid	80	91	3000 5%	150	0.512	0.500	2.39
7	Abortion	80	88	2000 5%	100	0.753	0.686	8.83
8	Ca. colon	54.6	115	1500 5%	75	0.926	0.832	10.00
9	Gang. appendix	86	103	2000 5%	100	1.13	0.82	27.34
10	Gast. neurosis	43	83	1000 10%	100	1.16	1.04	10.58
11	Pul. tubere.	63.4	97	1000 10%	100	1.193	0.871	26.98
12	Ca. colon	54.5	115	1000 10%	100	1.301	1.065	18.32
13	Rupt. appendix	61.8	95	3000 10%	150	1.323	1.132	14.43
14	Rectal fistula	57	90	1000 5%	50	1.46	1.43	2.16
15	Rupt. appendix	63	83	1000 5%	50	1.59	1.44	9.22
16	Intest. obstruct.	41.8	130	3000 5%	150	1.824	1.307	28.33
17	Alcoholism	68	..	1000 10%	100	1.960	1.953	0.367
18	Intest. obstruct.	48.1	130	3000 5%	150	2.216	1.565	29.40
19	Chr. glomerulo-nephritis	73	92	500 20%	100	3.600	3.537	1.885
20	Sept. abortion	46	91	500 20%	100	6.620	5.773	12.83
21	Chr. glomerulo-nephrosis	73	92	50 50%	25	20.5	18.661	9.66
22	Ca. pancreas	59	105	3000 5%	150	0.880	0.370	58.00

These observations have a practical application. It is customary to administer glucose solutions at the arbitrarily determined rate of about 60 drops a minute. This limit is supposed to guard against embarrassment of the circulation, and to obviate appreciable wastage of glucose in the urine. At 60 drops per minute a liter of fluid requires 333 minutes for injection (20 drops = 1 cc.). At the rate of 0.85 gm. of glucose per kilo per hour, a liter of 5% solution requires 60 minutes for injection (16 cc. per minute), a liter of 10% solution, 120 minutes (8 cc. per minute). These rates, for volume, are approximately those recommended as safe by Gilligan and Altschule,[3] 10 to 20 cc. per minute. The administration of 3 or 4 liters of fluid as commonly required to maintain fluid balance in fasting

and dehydrated adults consumes 15 to 20 hours at the 60-drop (3 cc.) per minute rate, five times as long as at 15 cc. per minute. It is our belief that the tedious drip method, often continued for 24 hours or longer, is needlessly tiresome and annoying to both patient and ward staff, and that practical considerations make it desirable to complete the parenteral administration of fluid during the daylight hours, as is possible in most cases. The studies of Gilligan and Altschule show this to be practicable, although due consideration must be given to circulatory weakness in individual cases, low plasma protein, weight of solution (5% glucose in physiologic saline is twice as heavy as either alone) and total volume to be injected. The present study shows that, although a portion of infused glucose is lost in the urine in all cases, this loss cannot be controlled by retarding the rate of infusion, and is, therefore, not an argument for drip administration.

Conclusions. The assimilation rate of intravenously administered glucose in hospital patients was found to be correlated with the rate of injection. The assimilation of 5.77 gm. per kilo of body weight per hour was observed.

In all cases some glucose was lost in the urine, the amounts varying from approximately 0.3% to 30% of the total given. The average loss was 12.2%.

The proportion of injected glucose lost in the urine was not correlated with the rate of injection, nor with the total amount given within the limits studied. This "spill" is conditioned by factors within the individual patients.

For practical purposes, the rate for the intravenous administration of glucose may be determined solely on two considerations: first, the hydrodynamics of the patient's circulation; second, technical convenience.

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FATAL HYPERINSULINISM WITH CEREBRAL LESIONS DUE TO PANCREATIC ADENOMA.

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Islet cell tumors of the pancreas are rather uncommon in autopsy material. Some 11 examples have been encountered among 6700 autopsies in this department since 1925. Usually these were quite small and were picked up only on routine microscopic section of the pancreas. The true incidence is therefore probably much higher

than this would indicate. Commonly they give rise to no symptoms, because either they do not secrete insulin, or, if they do, there is insufficient to upset the normal carbohydrate regulating mechanism. Approximately 100 cases have been described in the literature.[4] Four cases with surgical cure have previously been reported from this institution.[3]

The various symptoms resulting from hyperinsulinism are fairly well recognized and need not be recapitulated here. Wilder[7] has recently discussed them in detail.

The present case was thought worthy of record because of the rapid course to a fatal outcome, and the fact that the proper diagnosis was made and then discarded because glucose therapy did not afford the expected relief.

Case Report. N. D., a married woman, aged 42. Except for an unusual number of headaches in the previous 4 months, she was well until August 3, 1940, when she became mentally confused and wandered aimlessly about the house. She recovered spontaneously but the next morning remained in bed in a stuporous condition despite the fact that she was expecting guests for dinner. By 10 A.M. she had become perfectly normal again without treatment. The following day she was given a complete physical examination, but nothing abnormal was found. For some months she had been taking thyroid extract for obesity without her doctor's knowledge. In the next week she had two more spells of amnesia and confusion which passed off apparently without treatment; an adequate history of these was lacking.

On the morning of August 9, she was found lying in bed in a comatose condition from which she did not recover all day. Examination that evening by a consultant revealed only right lower facial weakness; the reflexes were normal and there was no papilledema. During the examination, she roused herself somewhat and it was noted that her speech was thick and blurred; all test objects were called by the same name. Because of strong resistance on the part of the patient an attempt at lumbar puncture was unsuccessful. A few minutes after the struggle, she suddenly regained all her faculties and was apparently normal. Later in the week she had several similar attacks during which she was incontinent; the complete history of this period was not available.

On August 13, 1940, she was admitted to the hospital after having been in a deep coma for the previous day and a half. Physical examination except for the coma and absence of all reflexes was negative. There was no response to any stimuli. The temperature was 103° F. and the pulse rate 108 per minute and regular. The spinal fluid was normal to routine examination; the blood N.P.N. measured 58 mg. per 100 cc.

A provisional diagnosis of hypoglycemia was made. The blood sugar at 3 P.M. equaled 43 mg. per 100 cc. After this was reported, she was given 100 cc. of 50% glucose in addition to the continuous intravenous of 5% glucose she had already been receiving. At 9 P.M. the clinical condition was not improved in spite of the fact that the blood sugar had risen to 172 mg. The next morning her temperature had fallen slightly but she was still deeply unconscious; the blood sugar had dropped to 66 mg.; 100 cc. of 50% glucose was again administered intravenously, but the coma continued and the blood sugar that evening was only 42 mg. On August 15 the temperature had risen to 104°, the blood sugar was 40 mg., and her clinical condition was unchanged. After several previously unsuccessful

attempts, a duodenal tube was passed and feedings given. The blood sugar had risen that evening to 93 mg. per 100 cc., but she was unimproved.

Up to this time the diagnosis of pancreatic adenoma with hyperinsulinism had been considered likely, but in view of the failure of intravenous glucose to rouse her from coma, though the blood sugar had been brought to normal or over on two occasions, this diagnosis was abandoned. It was thought that she might be suffering from an unusual form of encephalitis.

On August 17 the spinal fluid sugar was 73 mg. per 100 cc. Her condition remained unchanged until August 19 when the temperature rose to 106°, the pulse to 140 and she died 16 days after the beginning of the present illness and after 8 days of continuous coma.

Autopsy findings (complete autopsy 1½ hours after death). Gross: The body was well developed and rather obese, weighing 162 pounds. The only findings of interest were in the pancreas and the brain.

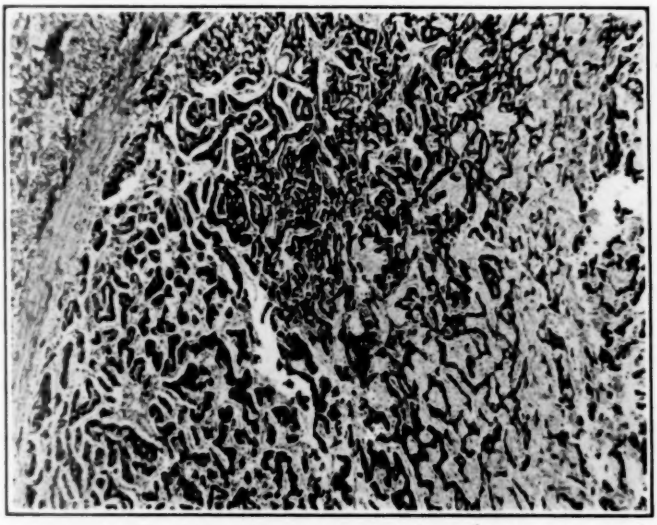

FIG. 1.—Larger islet cell adenoma of pancreas, including part of capsule and showing fibrosis of central portion. × 25. H. & E. (Low power.)

The pancreas weighed 65 gm. and measured 18 cm. in length. The configuration was normal except that in the tail, 3 cm. from the end, there was a small spherical nodule. About one-half of this mass protruded from the middle of the anterior surface of the pancreas. It was soft, well encapsulated and was dissected out easily; it weighed 2.7 gm. and measured 1.9 cm. in diameter. The cut surface was uniformly yellowish-white and not congested. Serial section of the rest of the pancreas revealed no lesions.

Assay of the insulin content of this tumor (Dr. D. A. Scott, of the Connaught Laboratories), showed 30 units of insulin per gm., or approximately 10 to 15 times the content of normal pancreatic tissue.

The brain weighed 1325 gm. There was congestion of the superior cerebral veins and a small quantity of fresh blood in the subarachnoid space over the right frontal lobe. The external surface showed no cortical atrophy or other abnormality. Section of the brain revealed some edema with narrowing of the ventricles but no other changes.

The pituitary weighed 0.6 gm. and was grossly normal.

The other organs were remarkably free from lesions.

Microscopic: Pancreas—the tumor was composed of epithelial cells arranged in slender ribbons, rounded masses and alveolar structures (Fig. 1). The arrangement varied in different fields but was chiefly of the ribbon type; often these ribbons were made up of a single layer of cells, but many also resembled pseudostratified columnar epithelium. The alveolar structures consisted of a single row of columnar cells around a capillary, only occasionally forming a complete circle. It is possible that these differences in appearance were due to the cutting of very tortuous ribbons at various levels and in different directions.

With hematoxylin and eosin stain some of the epithelial cells had large round nuclei which tended to be vesicular in type, but most of them were smaller and oval in shape and the chromatin was more diffusely distributed (Fig. 2). Usually the nuclei were centrally placed; the oval nuclei were often squeezed slightly to one pole by the pressure of contiguous cells. In the alveolar structures the nuclei were nearly always peripheral. In gen-

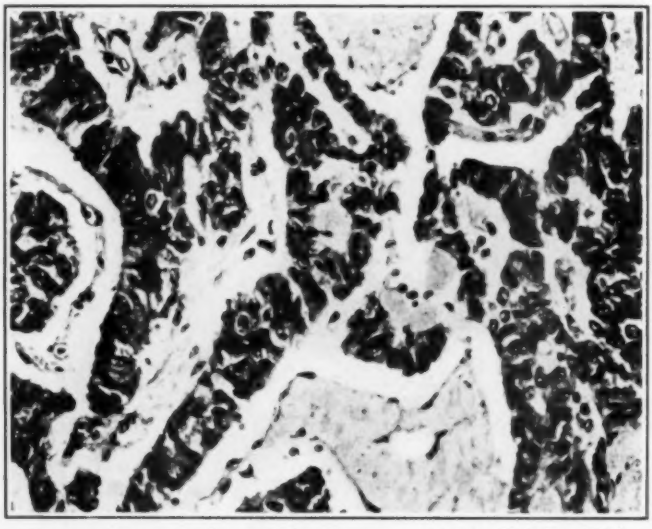

FIG. 2.—Larger adenoma to show cellular structure. × 240. H & E. (High power.)

eral appearance most of these cells did not closely resemble normal islet cells, but near the capsule some did, the nuclei being smaller and more diffusely stained. Mitotic figures were rare and there was no invasion of vessels. The cytoplasm was finely granular and purplish in color.

Surrounding these columns of cells but separated from them by an open space was a fine supporting network of naked capillaries usually having a diameter about that of a red cell. In many places there appeared to be openings in these capillaries and a number of red cells lay free in the spaces next to the epithelial masses. Near the capsule the framework was thicker and there were fibrocytes surrounding the capillaries; a number of lymphocytes were also seen. Toward the center of the tumor the cell cords were separated and compressed by irregular and relatively acellular masses of connective tissue surrounding the capillary network. In a few areas these showed beginning hyalinization; no calcification was evident. The occasional small pancreatic duct radicle was observed.

The capsule of the tumor was composed of a layer of collagenous connective tissue of variable thickness in which lay a number of islets and small ducts. These islets were somewhat flattened but appeared normal except that some were several times the usual size.

Though special stains of the formalin-fixed tissue were not entirely satisfactory, Bowie's stain did show that about 75% of the granules appeared to be of the beta type. Most cells contained only beta granules; many contained alpha granules as well.

Routine blocks of the pancreas elsewhere revealed another small adenoma about half the size of a low power field (Fig. 3). The general structure was the same as that of the larger tumor except that the capsule was thin and in some areas deficient, and there was much less fibrosis.

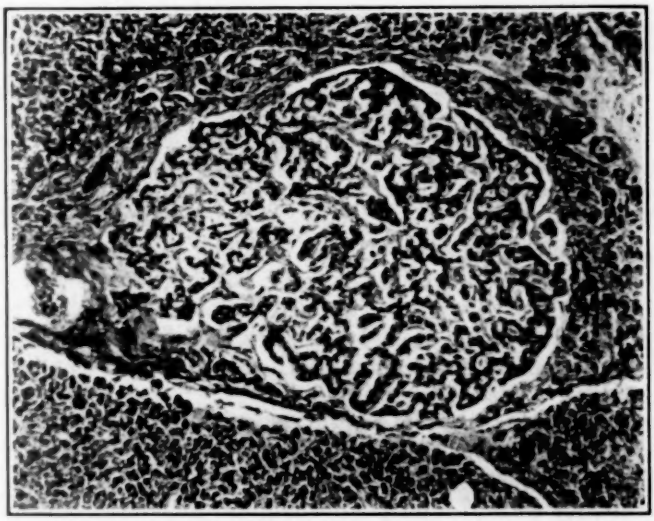

FIG. 3.—Smaller adenoma showing resemblance to the larger one. × 70. H. & E. (Low power.)

Several other sections of the pancreas presented no abnormality.

Brain. Numerous blocks were cut and stained with hematoxylin and eosin, cresyl violet, Mallory's phosphotungstic acid and Mallory's connective tissue stain.

In the left frontal lobe there was much thickening and gliosis of the molecular cortical layer. Edema was well marked in both the gray and white matter and there was considerable swelling of oligodendroglia. The small vessels were congested and there was hemorrhage into the perivascular spaces in some areas; in others a few lymphocytes were seen. Some of the cortical nerve cells showed pyknosis and shrinkage. In the deeper layers of the cortex neuronophagia was evident. In the other sections of the cerebrum, similar changes were present in varying proportions. The left hypothalamus presented subependymal gliosis, congestion and an occasional perivascular hemorrhage; no significant nerve cell changes were noted.

In the midbrain there was moderate subependymal gliosis below the aqueduct and just deep to the pia. Edema and congestion were evident here also and there was some swelling and chromatolysis in the nerve cells of the oculomotor nucleus.

In the pons the ependyma was thrown into folds by proliferation of underlying glial tissue; gliosis was also evident around the small vessels. Many of the nerve cells revealed acute degenerative changes with swelling of the cell bodies and eccentration of the nuclei.

Transverse section of the open medulla showed an intact ependyma beneath which lay a narrow band of gliosis. Edema and congestion were evident throughout the tissue. In the superficial portions of the floor of the fourth ventricle a few of the nerve cells showed pyknosis but the majority were entirely normal.

The *arteries* and arterioles were everywhere normal.

Pituitary. The gland was preserved in formol-saline and cut transversely. Sixteen pairs of slides were made at various levels and stained with hematoxylin and eosin and Mann's stain. There was some variation in the picture from section to section. The normal proportions of the various cell types were present in several sections, but on the whole the eosinophils were less numerous than usual. The number of chromophobes was definitely increased in many places. The basophils were slightly increased in some sections but this was not a prominent feature. No basophil adenomata were seen; two small, poorly circumscribed, nodular masses of chromophobe cells were identified. The pars intermedia presented no abnormality. Slight invasion of the posterior lobe by basophils was evident in some sections but this never exceeded normal limits.

Microscopic examination of the *other organs* revealed no abnormalities.

Comment. The majority of cases of islet cell tumor run a prolonged intermittent course which may last for some years. One case, reported by Barnard,[2] died about 4 months after the onset of symptoms, but I have not encountered in the literature a case comparable to this in which the total length of the illness was apparently only 16 days.

In this patient stupor and mental confusion were the outstanding features, and the attacks came on without the prodromal symptoms and signs so frequently seen. The severity of the process probably accounted for this. Motor phenomena except for facial weakness on one occasion and speech defect were notably absent.

One attack terminated spontaneously just after a struggle induced by attempted lumbar puncture. One may postulate that the physical effort resulted in release of adrenalin with mobilization of glucose from the liver.

Wilder,[7] quoting Whipple, states that the relief of symptoms and return to normal resulting from the administration of glucose is one of the criteria for the diagnosis of hyperinsulinism. Usually this criterion can be fulfilled, but unfortunately in the present case the strict application of this principle caused the abandonment of the correct diagnosis. The effect of glucose is generally dramatic, but if the attack is very severe and prolonged no clinical improvement may result.

In the temporary episodes quickly and completely relieved by glucose, it seems not unlikely that the hypoglycemia is *per se* responsible for the symptoms, as it apparently is in hypoglycemia from causes other than hyperinsulinism. In the severe attacks whose sequelæ may be hemiplegia, idiocy, dementia, and so on, and

in which bringing the blood sugar to or above normal does not restore the patient, cerebral damage is probably the main factor.

The cerebral changes produced by fatal hyperinsulinism whether due to islet cell tumor or insulin shock therapy are variable. They have recently been reviewed by Malamud and Grosh,[6] and Baker.[1] Congestion, edema, hemorrhages, perivascular lymphocytic infiltration, cortical atrophy, degeneration of ganglion cells in cortex and basal ganglia, degeneration of neurones and encephalomalacia have been described by different authors. Similar lesions have been produced in the experimental animal in some cases.

In this case, moderate edema and congestion, and a small subarachnoid hemorrhage were present in the gross. Microscopically, the cerebral cortex showed some degeneration of nerve cells and neuronophagia. Nerve cell changes were also seen in the mid-brain and pons but not in the hypothalamus. The rapid progress of the disease would seem to account for the lack of striking changes in the brain. The gliosis, though not marked, is difficult to explain. It is unlikely that it could have developed in just over 2 weeks. Nor is it probable that it represents pre-senile changes in so young a woman, especially in view of the complete absence of vascular sclerosis. An alternative explanation is that the patient suffered a number of mild hypoglycemic attacks which passed unnoticed. It cannot be denied for certain that the headaches over a period of 4 months preceding the apparent onset of the illness did not represent hypoglycemic episodes; if they did, they would seem to have been too slight to cause cerebral damage.

The mechanism of insulin action on brain tissue has not been satisfactorily explained. A number of theories have been advanced, but no adequate evidence for any of them has been adduced.

Several authors have drawn attention to changes in the pituitary gland. Friedman[5] has recently described basophil and eosinophil hyperplasia of the anterior lobe in 2 cases, 1 of which also showed massive basophil invasion of the posterior lobe. Malamud and Grosh[6] mentioned in their case that there was eosinophil hyperplasia of the anterior lobe and a small basophil adenoma. No alterations of the pituitary have been consistently described and, in most cases, the gland has been normal. Those changes present in this case were not of marked degree and did not correspond to those noted by Friedman. Their significance, if any, is not clear.

Summary. A case of fatal hyperinsulinism due to multiple islet cell tumors of the pancreas has been reported. The patient died 16 days after the onset of the first symptoms, and after 8 days of continuous coma.

The tumor contained 30 units of insulin per gram of tissue.

The brain showed congestion, edema, hemorrhages, nerve cell degeneration and gliosis, but these changes were not severe.

The case was notable on account of the rapidly fatal issue and the

fact that the diagnosis was missed because the patient did not respond to the administration of glucose.

I am indebted to Prof. William Boyd for permission to publish this case and to Prof. Eric Linell for the use of his reports on the brain and help in their interpretation.

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THE PROGNOSIS OF PNEUMOCOCCIC MENINGITIS TREATED WITH CHEMOTHERAPY.

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With the growth, popularity and intelligent use of the newer methods of therapy in pneumococcic meningitis the prognosis has been much improved. Following our first successful recovery of a Type XIV pneumococcic meningitis we became sufficiently interested to review the literature in order to determine the value of chemotherapy in this infection. This report is a review of the 260 reported cases that have been treated with chemotherapy, with or without specific serum, in an attempt to evaluate the effectiveness of each of the therapeutic agents, alone and combined.

Table 1.—Results of Treatment of Pneumococcic Meningitis in All Reported Cases.

	Total.	Died.		Recovered.	
		No.	%.	No.	%.
Sulfanilamide	78	50	64.1	28	35.9
Sulfanilamide—serum	50	34	68.0	16	32.0
Sulfapyridine	82	34	41.5	48	58.5
Sulfapyridine—serum	50	21	42.0	29	58.0
Chemotherapy	160	84	52.5	76	47.5
Total	260	139	53.5	121	46.5

Chemotherapy. In the years previous to the use of the sulfonamide drugs the number of cures (63) were so few that the fatal prognosis in this disease was considered to be from 95% to 100%. From Table 1 it is seen that in 160 cases treated with chemotherapy alone, sulfapyridine bears out experimental evidence that it is a more efficacious drug in pneumococcic meningitis than is sulfanilamide. In 82 cases treated with sulfapyridine there were 58.5% recoveries compared to the recovery rate of 35.9% in 78 cases treated with sulfanilamide.

Other sulfonamide drugs have to be considered as possible therapeutic agents. While a recovery with the use of sulfathiazole has been reported[25] its poor concentration in the spinal fluid compared to its concentration in the blood eliminates it as a factor in the treatment of this infection. Sulfadiazine (2-sulfanilamidopyrimidine) has been shown to pass over into the cerebrospinal fluid[22,23] in concentrations of two-thirds to four-fifths of that which exist in the blood. While experimentally[12] it is less effective in Type I pneumococcic infections in mice, clinical evidence may show it to be of some value in this disease.

Chemotherapy and Specific Serum. It has been definitely stated by many that there exists no clear evidence that the use of serum with the sulfonamide drugs has materially affected the course of pneumococcic infections.[3,10,17,19,29] Others[2,4,5,7,15] have found both experimentally and clinically that the efficiency of specific serum as well of the sulfonamide drugs, was enhanced by the use of the two simultaneously. In these studies this action was beyond that which would be expected from the simple additive effect of the two.

Table 1 shows that the percentage of recovery with sulfapyridine as compared to sulfapyridine-serum therapy is similar, 58% recoveries. In Types I, V, and VII in which the value of serum is the most efficacious, Table 2 shows that there is little difference in the fatality rates from drug or drug-serum therapy with the exception of Type V, in which combined therapy is of value (83% recovery with sulfapyridine-serum therapy). However, combined sulfapyridine-serum therapy may raise the prognosis in the younger age groups (Table 4), and in meningitis secondary to an otorhinologic condition (Table 3).

It has been suggested that both specific serum and sulfapyridine be administered in adequate dosage as early as possible for its immediate effect in tiding the patient over the immediate emergency since Haviland[9] has shown that with chemotherapy alone antibodies may not occur for several days after the temperature has fallen to normal, while MacIntosh and Whitby[13] demonstrated that it takes 6 hours or more for the bacteriostatic action of sulfapyridine to take place. Contrary to this, Spring and his associates[26] demonstrated that in some patients even the presence of a high titer of antibodies does not guarantee recovery, while recovery may occur in others without demonstrable antibodies in the blood stream. Furthermore the combined drug-serum therapy may be given to the younger age groups, in meningitis secondary to an otorhinologic condition, in patients who have an idiosyncrasy to the sulfonamides in an effort to lessen toxic effects, in those who fail to respond within 24 to 48 hours to chemotherapy and in those cases in which the pneumococcus develops a tolerance to the drug.

Dosage. The doses of the sulfonamide given to recovered cases has been quite variable. MacKeith[14] has reported a recovery with

a total dose of 12 gm. of sulfapyridine while Rhoads[24] has had to use a dose as high as 176 gm. throughout the illness for recovery to occur. Fatal cases have received doses as high as 90 gm. without appreciable effect.

TABLE 2.—TYPES OF PNEUMOCOCCUS IN REPORTED CASES OF PNEUMOCOCCIC MENINGITIS WITH THE USE OF SULFONAMIDE DRUGS.

Type.	Sulfanilamide.		Sulfanilamide serum.		Sulfapyridine.		Sulfapyridine serum.	
	Died.	Recovered.	Died.	Recovered.	Died.	Recovered.	Died.	Recovered.
I	3	2	0	2	1	3	2	3
II	0	0	0	2	0	0	0	0
III	10	8	3	1	4	4	3	6
IV	2	2	0	1	1	3	3	3
V	1	3	0	0	0	3	1	5
VI	3	0	1	2	3	0	0	0
VII	3	0	0	4	0	0	1	0
VIII	0	0	1	0	0	0	1	0
IX	1	0	0	0	0	1	1	0
X	0	0	0	0	3	1	1	1
XI	0	0	1	0	0	0	0	0
XII	0	1	0	0	1	1	0	0
XIII	1	1	0	0	0	0	0	1
XIV	0	1	0	0	2	2	2	3
XV	0	1	0	0	0	1	0	0
XVI	0	0	0	0	1	0	0	0
XVII	0	1	0	1	1	1	0	2
XVIII	1	0	2	0	4	2	3	0
XIX	1	0	0	1	2	2	0	2
XX	0	1	0	0	0	0	0	1
XXI	0	0	1	0	0	0	1	1
XXII	0	0	0	0	1	0	1	0
XXIII	0	0	0	0	1	2	1	0
XXIV	0	0	0	0	0	0	0	0
XXV	0	0	1	0	0	2	0	0
XXVI	0	0	0	0	0	0	0	0
XXVII	0	1	0	0	1	0	0	0
XXVIII	0	1	1	1	0	1	0	0
XXIX	0	2	0	0	0	0	0	0
XXX	0	0	0	0	0	0	0	0
XXXI	0	1	0	1	1	0	0	0
XXXII	0	0	0	0	0	0	0	1
Over XXXII	2	0	0	0	1	1	0	0
Not stated	22	2	23	0	6	18	0	0
Total	50	28	34	16	34	48	21	29

The difficulty in regard to dosage is the irregularity of absorption of the drug, thus accounting for the great variations in its effect and in its concentrations in both blood and spinal fluid. In this respect while a level of sulfapyridine ranging from 10 to 20 mg. per 100 cc. of blood is desirable, Hodes[11] has reported recoveries with blood levels as low as 1.7 mg. per 100 cc. in some, to as high as 30.3 mg. per 100 cc. in others; with spinal fluid levels ranging from 1.1 mg. per 100 cc., to as high as 29 mg. per 100 cc.

The time required for the cerebrospinal fluid to become sterile varies considerably both in regard to the concentration of the drug and the dosage. Generally in the majority of recovered cases it

becomes sterile in 4 days or less. A sterile culture obtained within this time was only rarely followed by death. In those cases with relapse, during which time the cerebrospinal fluid again contained pneumococci, the time taken to sterilize the spinal fluid also varied, but averaged about 7 days.

TABLE 3.—ETIOLOGIC INCIDENCE OF REPORTED CASES OF PNEUMOCOCCIC MENINGITIS WITH RECOVERY COMPARED TO INCIDENCE OF TOTAL REPORTED CASES.

	Av.* incidence.	Non-specific treatment.	Specific serum.	Sulfanilamide.	Sulfanilamide and serum.	Sulfapyridine.	Sulfapyridine and serum.
Otitis media and/or mastoiditis	101 34.8%	8 28.5%	1 2.9%	15 53.6%	3 18.8%	14 29.2%	14 48.1%
Sinusitis	14 4.8%	1 3.6%	0	3 10.7%	3 18.8%	3 6.2%	1 3.5%
Head injuries	22 7.5%	1 3.6%	6 17.1%	2 7.1%	1 6.2%	1 2.1%	1 3.5%
Operations on head	8 2.7%	1 3.6%	1 2.9%	3 10.7%	0	2 4.2%	1 3.5%
Pneumonia	49 16.9%	5 17.9%	9 25.8%	0	1 6.2%	3 6.2%	2 6.9%
Upper respiratory infections	18 6.2%	5 17.9%	6 17.1%	1 3.6%	0	2 4.2%	2 6.9%
Primary	65 22.3%	6 21.3%	12 34.2%	3 10.7%	8 50.0%	15 31.3%	7 24.1%
Miscellaneous	14 4.8%	1 3.6%	0	1 3.6%	0	8 16.6%	1 3.5%
Total	291	28	35	28	16	48	29

* Total cases of authors quoted.[6,8,20,21,24,27]

Type of Pneumococcus. Investigation with regard to the types of pneumococci which are involved in pneumococcic meningitis, as given in Table 2, show the wide variety which are responsible in this infection. The effect of the sulfonamides upon the various types shows that their efficiency is relatively unimpaired by the type of pneumococcus involved. This is in accordance with the experimental work of Whitby[28] and Maclean[15] who have both shown that while pneumococci vary enormously in their sensitivity to the sulfonamides, the variation depends not on the type but on the individual strain. With the exception of Types III and V pneumococcic meningitis, there is little correlation, at least at present, between the types of pneumococci and whether sulfonamides alone or combined with serum are used. In these two types the combined sulfapyridine-serum therapy seems to be indicated.

Etiology. Pneumococcic meningitis occurs as a secondary complication in a number of disorders, including middle-ear or mastoid disease, pneumonia, paranasal sinusitis, upper respiratory infections, endocarditis, brain abscess, pneumococcic peritonitis and pleuritis. It most frequently occurs secondary to an otorhinologic condition, 34.8% of all cases, followed next in frequency by the primary involvement of the meninges, shown in Table 3 to occur in 22.3%.

In the 184 recoveries given in Table 3 the incidence of spontaneous

recoveries closely follows its occurrence as a whole. Serum therapy shows poor results in meningitis secondary to otorhinologic conditions, possibly due to the fact that there was inadequate treatment of the primary focus. This is very much offset by the use of chemotherapy, where with drug alone or drug-serum therapy there is approximately 50% recovery.

TABLE 4.—AGE INCIDENCE IN CURED CASES OF PNEUMOCOCCIC MENINGITIS IN COMPARISON TO AVERAGE INCIDENCE.

Age.	Incidence.*	Non-specific therapy.	Serum.	Sulfanilamide.	Sulfanilamide and serum.	Sulfapyridine.	Sulfapyridine and serum.
Up to 1 yr.	33	4	0	0	0	0	3
	14.1%	13.8%					10.3%
1 to 5 yrs.	44	6	3	5	0	1	4
	18.7%	20.7%	8.6%	17.9%		2.1%	13.8%
5 to 10 yrs.	33	5	5	6	4	8	4
	14.1%	17.2%	14.3%	21.4%	25.0%	16.5%	13.8%
10 to 20 yrs.	29	6	8	8	8	9	9
	12.3%	20.7%	22.9%	28.6%	50.0%	18.8%	31.0%
Over 20 yrs.	90	8	18	7	4	23	7
	38.3%	27.6%	51.4%	25.0%	25.0%	47.9%	24.2%
Not given	6	0	1	2	0	7	2
	2.5%		2.8%	7.1%		14.6%	6.9%
Total cases	235	29	35	28	16	48	29

* As given by Neal.[18]

PROTOCOL.—CASES TREATED AT JEFFERSON HOSPITAL WITH CHEMOTHERAPY.

Sex.	Age.	Type of pneumococcus.	Source of infection.	Treatment.	Remarks.
F	56	*	Primary	Spinal taps; sulfanilamide; azosulfamide	Diabetic patient with diabetes under control; died 24 hrs. after admission.
M	6 mos.	VI	Primary	Spinal taps; sulfanilamide	Died 6 days after admission; spinal fluid never sterile.
F	18 mos.	IX	Pneumonia	Spinal taps; azosulfamide; optochin	Patient died on 8th day; pneumococci obtained from spinal fluid daily except on 8th day.
M	6 mos.	VI	Pneumonia	Sulfapyridine	Patient died 36 hrs. after admission.
M	8	III	Otitis media	Spinal taps; sulfapyridine (orally, I.V.)	No signs of meningitis 1 mo. after admission; relapsed in 6 wks. and died; brain abscess on autopsy.
M	5	XIV	Primary	Specific serum; sulfapyridine (oral, subcut., I.V.)	Spinal fluid sterile on 3d day; patient recovered.
M	38	XVIII	Primary	Spinal taps; specific serum; sulfapyridine	Patient died on 4th day; spinal fluid culture positive.
M	21	X	Foll. skull fracture	Spinal taps; specific serum; sulfapyridine	No signs of meningitis 6 wks. after admission; relapsed, died in 3 days.
F	2 mos.	V	Otitis media	Sulfathiazole	Patient died within 24 hrs. of admission.

* Type not determined.

The poorer prognosis shown in pneumococcic meningitis arising secondary to pneumonia is worth noting. It has already been shown[15,28] experimentally that there occurs an acquired tolerance of the pneumococci to sulfapyridine. This has been shown clinically by May[16] and by Auger[1] who with pneumococci cultured from the spinal fluid of a case of pneumococcic meningitis showed that the strain of pneumococcus cultured from the spinal fluid before treatment was sensitive to sulfapyridine while the same strain cultured

from the fluid after 29 days of intensive treatment showed itself to be resistant to sulfapyridine. A further possibility is that in those pneumonia patients in whom the organism becomes resistant to sulfapyridine and the disease spreads to involve the meninges the prognosis is poor and here combined drug-serum therapy should be used.

Age. While pneumococcus meningitis occurs in all age groups, Table 4 shows it to occur more frequently in children. Most of the recoveries obtained with specific serum have occurred in adults, while the use of chemotherapy and still better sulfapyridine and serum combined give a better prognosis for cure in the younger age groups. This combination gives the best prognosis in the ages between 10 and 20 years in which almost one-third of the recoveries have occurred.

Summary and Conclusions. 1. The clinical results obtained in the treatment of 160 reported cases of pneumococcic meningitis with chemotherapy bear out experimental results that sulfapyridine is the drug of choice in the treatment of this infection.

2. At present there is no clear evidence that the additional use of specific serum with sulfapyridine should be a routine procedure. The percentage of recovery with sulfapyridine alone is approximately that of combined sulfapyridine-serum therapy. The combination is indicated in the treatment of Type III and V pneumococcic meningitis, in those infections secondary to an otorhinologic condition and to pneumonia, and in the younger age groups.

3. No definite correlation could be made between the dose of the sulfonamide drug used, its concentrations in both blood and spinal fluid and the clinical response.

4. The prognosis is favorable if the cerebrospinal fluid has been sterilized within 4 days with the use of sulfapyridine.

5. Almost every type of pneumococcus has been cultured from cases of pneumococcic meningitis. With the exception of Types III and V, there is little correlation between the type of pneumococcus and the prognosis.

6. The prognosis in pneumococcic meningitis secondary to pneumonia is poor, possibly due to an acquired tolerance of the invading organism to the sulfonamide drug.

7. The prognosis in the younger age groups is improved with the use of combined drug-serum therapy, with the best prognosis occurring in the ages from 10 to 20 years.

8. In 9 cases of pneumococcic meningitis treated at Jefferson Hospital with chemotherapy, only 1 recovered. Two other cases, Types III and X, had apparently recovered but died following unsuccessful treatment of a relapse. The recovered case received combined sulfapyridine-serum therapy.

The author wishes to thank Dr. E. L. Bauer, Professor of Pediatrics, for his help in compiling this report, and Dr. H. A. Reiman and Dr. B. J. Alpers for permission to use cases on their ward services.

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PNEUMOCOCCAL CAPSULAR POLYSACCHARIDE AND ANTIBODY IN PLEURAL EXUDATES.*

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Studies on the diagnostic and prognostic significance of the detection of pneumococcal capsular polysaccharide ("SSS") in blood and urine of patients with pneumococcal pneumonias have been reported.[1,3,4,8,9a,b] We record in this study from the Pneumonia Service at Harlem Hospital during the season of 1939–40 the occurrence of SSS, and of agglutinative and precipitative antibodies in pleural exudates.

* This study received additional financial support from the Metropolitan Life Insurance Company, and from Mr. Bernard M. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mrs. Robert H. Samstag.

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An extensive bacteriologic study of pleural effusions was first published about 50 years ago, by Netter,[16] and more recently, in 1930, by Locke[15] who showed that pneumococcus I was the most frequent cause of empyemas.

Studies on antibodies in pleural effusions were made by Griffon in 1899.[13] He found that pneumococci grown in a pneumococcic pleural exudate might become agglutinated. Cole,[5] in 1917, found that pleural empyema fluids inhibited the protective power of homologous antipneumococcal sera, and did not contain agglutinins or protective substances. More recently, in 1932, Finland[10] published immunologic studies on 31 specimens of pleural fluid from 19 patients with typical lobar pneumonias. He found SSS only in infected fluids and antibodies only in sterile fluids.

Several other immunologic studies concerning the bactericidal properties of pleural exudates have been made.[6a,7,11,12,17–22] In these studies all the fluids accompanied by pleural inflammation were bactericidal for the organisms tested, including the pneumococcus.

In the present series pleural exudates of patients with typical lobar pneumonias were examined bacteriologically and immunologically (detection of SSS, agglutinins and precipitins) and the findings correlated with other bacteriologic, immunologic and clinical data.

An attempt was made to determine the pneumococcic type responsible for an illness by identifying with pooled serums the specific capsular polysaccharide in the pleural exudate.

The "optimal" concentration of antibody required for the detection of SSS in pleural exudates was determined for some pneumococcic types.

Material and Methods. A total of 44 specimens of pleural exudate from 27 patients with lobar pneumonias were examined. Forty-three samples from 26 patients were due to pneumococci; one exudate was obtained from a patient suffering from a *Bacillus Friedlander A* pneumonia. For controls, 4 pleural fluids (from 4 patients) with diseases other than pneumonia were studied. Venous blood and urines were examined in some of the patients.

Detection of Capsular Polysaccharide in Pleural Exudates.* The method of determining capsular polysaccharide by type-specific or by pooled sera, and the method of determining antibodies have been already described in previous publications[2,3,6b] in studies with blood and urine.

One-half milliliter of the undiluted clear supernate, obtained by centrifugation, of the pleural fluid was mixed with 0.5 ml. of the homologous rabbit antiserum in appropriate dilution; for controls 0.5-ml. portions of the same pleural fluid were mixed in a second and in a third tube with 0.5 ml. of a heterologous rabbit antiserum and with 0.5 ml. of saline respectively. The two controls always yielded negative results. In a few cases the undiluted supernate of the pleural fluid contained such large amounts of SSS that no reaction with the type-specific antiserum was observed. Precipitates appeared, however, when the tests were repeated with 1:2, 1:4, and 1:8 dilutions of the pleural exudate.

* The capsular polysaccharide and the serum used were those commercially available or supplied by the Lederle Laboratories, Inc., and were used in our previous studies.

TABLE 1.—BACTERIOLOGIC AND IMMUNOLOGIC STUDIES OF PLEURAL EXUDATE AND OTHER CLINICAL AND LABORATORY OBSERVATIONS.

Case No.	Type.	Age and sex.	Lobe involved.	Day of disease.				Serum treatment, units and kind.	Sulfapyridine, total gm.	Blood.				Urine, SSS.	Pleural exudate.				Outcome.
				Serum. Started.	Drug. ted.	Thoracentesis. Done.	Thoracotomy. ne.			Culture.	SSS.	Agg.*	Prec.*		Culture.	SSS.	Agg.*	Prec.*	
1	I	31 F	R.L.	..	9	30	39	...	66	0	0	64	0	+	+	++++B	0	0	Recovered
2	I	5 M	R.U.	..	6	37	43	..	20	0	..	..	..	..	+	++B	0	0	Recovered
3	I	35 M	R.M. R.L.	6	..	18	6	340,000 R	...	0	0	..	..	..	+	+++B	0	0	Recovered
4	II	60 M	L.L.	4	..	9	..	265,000 H	..	0	0	32	8	..	+	+	0	0	Recovered
5	III	56 M	L.L.	..	?	3h 9h 11h 15h 23h	18h	...	67	0	+	0	0	+	+ + + + +	+B +B ++B ++B ++B	0 0 0 0 0	0 0 0 0 0	Died
6	IV	27 M	L.U. L.L.	3	..	3	..	259,000 R	..	+	..	..	..	..	+	+B	0	0	Died
7	V	32 F	R.L.	?	?	10h 11h 15h	21h	437,000 R	95	0	..	..	..	..	+ + +	++B ++B +B	0 0 0	0 0 0	Recovered
8	V	58 M	L.L.	..	8	23	32	..	79	+	0	0	0	0	+	±P	0	0	Recovered
9	VII	30 F	R.L.	7	..	17 21	21	640,000 R	..	+	0	64	16	0	+ +	+B +B	0 0	0 0	Recovered
10	VIII	45 M	L.L.	3	6	24	28	463,750 H and R	57	0	..	..	..	0	+	++B	0	0	Recovered
11	VIII	23 M	L.L.	4	4	11	13	177,750 R	23	+	0	..	..	..	+	+++B	0	0	Died
12	VIII	58 M	R.L.	6	4	7	..	200,000 R	30	+	0	64	16	..	+	+++B	0	0	Recovered
13	XIV	56 M	R.L.	.	9	35 40	43	...	87	+	0	64	8	..	+ +	+++B ++P	0 0	0 0	Recovered
14	II (Landsteiner A	37 M	L.L.	..	3	13	..	..	64	0	0	..	8	..	+	++++B	0	0	Recovered
15	II	24 F	L.L. R.L.	4	4	12 60	..	535,000 H and R	28	+	0	64	16	.	0 +	0 ++B	0	0	Recovered

12	XIII	28	M																
13	XIV	56	M	R.L.	..	9	35 40	43	....	87	+	0	64	8		+ +	++P	0	0	
14	II Flander A	37	M	L.L.		3	18			64	0	0		8		+	+ + + + B	0	0	Recovered
15	II	24	F	L.L. R.L.	4	4	12 60	..	535,000 H and R	28	+	0	64	16	..	0 +	0 ++B	0	0	Recovered
16	III	47	M	R.L R.U.	3	3	9 10 12 14	17	332,500 R	57	0	+	32	16	..	0 0 + 0	*B *B +B ++B	0 0 0 0	0 0 0 0	Died
17	III	49	M	R.U.	..	3	9	..	...	25	0	0	4	0	+	0	+B	0	0	Died
18	VII	39	M	R.U.	5	..	18	..	322,000 R	....	+	0	..	..	..	0	*P	0	0	Recovered
19	II	58	M	L.L. R.L.	10	8	7	..	518,000 R	44	+	0	..	..	+	0	+B	0	0	Recovered
20	VIII	43	M	R.L. R.M. ?	1	1	10	.	186,400 H	14	+	+	64	16	+	0	0	0	0	Recovered
21	XII	30	F	R. L.	..	6	16	..	...	17	0	..	..	..	..	0	0	0	0	Recovered
22	III	43	M	L.L.	2	2	12	..	300,000 R	21	0	0	0	0	..	0	0	0	0	Recovered
23	XXIX	29	F	L.L.	..	?	5h	..	...	132	0	..	..	..	0	0	0	0	0	Died
24	XIII	26	F	R.L.	15	15	13 24 30 43	..	625,000 R	18	0	0	16	16	..	0 0 0 0	0 0 0 0	0 4 64 0	4 8 8 0	Recovered
25	V and VIII	53	M	L.L.	4	12	14	..	237,000 R (VIII) 280,000 R (V)	22	0	..	..	..	..	0	0	16 (V)	0	Recovered
26	I	26	F	L.L.	..	3	18 19	..	...	32	0	0	..	..	..	0 0	0 0	8 4	0 0	Recovered
27	I	61	M	R.U.	13	3	14 15	..	381,500 R	67	+	..	..	..	..	0 0	0 0	8 4	0 0	Died

h, day of hospitalization (? day of disease); P, precipitate; B, button; H, horse serum; R, rabbit serum.

* The figures given for agglutination and precipitation indicate the highest final dilution of the material under investigation (blood, pleural exudate) where positive reactions were observed.

Detection of Agglutinins. One-half-milliliter quantities of serial dilutions, carried to $\frac{1}{32}$, of the supernate of the pleural exudate, were mixed with 0.5 ml. of the saline suspension of heat-killed homologous pneumococci.

Detection of Precipitins. One-half-milliliter quantities of serial dilutions, carried to $\frac{1}{4}$, of the supernate of the pleural exudate, were mixed with 0.5 ml. of an appropriate dilution of homologous SSS. A control tube, containing 0.5 ml. of the pleural exudate with 0.5 ml. of a heterologous SSS solution was negative in all the experiments. The tests were always made in 10 by 75 mm. Pyrex tubes by the centrifugation method[6b] at 2000 r.p.m. for 30 minutes. Positive results varied from a cloud ("+") to a precipitation or button formation of varying intensity ("±p" to "++++B").*

Controls. Further controls included the following mixtures: 1, type-specific serum plus type-specific SSS; 2, type-specific serum plus saline; 3, heterologous serum plus its type-specific SSS; 4, heterologous SSS plus saline; 5, homologous SSS plus saline; 6, hererologous SSS plus saline; 7, type-specific serum plus bacterial suspension; and 8, saline plus bacterial suspension. The controls always yielded the expected results (positive in tubes Nos. 1, 3 and 7; negative in Nos. 2, 4, 5, 6 and 8).

Results. The results of the bacteriologic and immunologic studies of the pleural exudates, and other clinical and laboratory observations are recorded in Table 1. The 44 samples of pleural fluid from 27 patients may be divided into three groups. The first group of 28 fluids (Patients 1 to 16) includes those having no antibody but containing pneumococci and SSS either on the first or on subsequent study. The second group of 3 fluids (Patients 17 to 19) were negative on culture and for antibodies but contained SSS. In the third group of 13 fluids (Patients 20 to 27) neither organisms nor SSS were detected but exudates from 4 of the patients contained antibodies on one or more occasions. In 3 of these, agglutinins only, and in 1, both agglutinins and precipitins were evident. A total of 6 pleural fluids from 4 patients (Nos. 16, 17, 18, 19) contained SSS, though sterile. These results indicate that the detection of SSS in "sterile" pleural effusions may reveal the pneumococcus type involved.

Table 2 records the deaths for each of the three groups, the bacteremic incidence and the relationship between the presence of SSS in the pleural exudates and in the blood. The number of cases are too few to permit statistically valid conclusions. The mortality rate of the group is 29.7% (8 deaths among the 27 patients). This table also indicates the desirability of performing various tests on body fluids, because SSS may be found in the blood when no organisms can be detected (2 patients of the first group).

Table 3 confirms this observation and shows the relationship between the determination of SSS in pleural exudate, urine, and blood.

The type distribution was found to be fairly scattered with pneumococcus Type I the most frequent (5 cases).

Of the 27 patients, 5 were treated with serum only and 1 died,

* In subsequent experiments performed by Sonia Levine in this laboratory, in two pleural fluids containing viable organisms and soluble carbohydrate the button obtained on adding serum to pleural fluid contained agglutinated organisms.

10 received sulfapyridine only and 3 died, and 12 received both serum and sulfapyridine, 4 of whom died. In the 4 cases where antibodies developed, 1 case (No. 27) died and in this case only agglutinins were found. The other 3 patients recovered, in 2 only agglutinins were present, and in the third both agglutinins and precipitins.

TABLE 2.—RELATIONSHIP BETWEEN PRESENCE OF ORGANISM AND SSS IN BLOOD AND PLEURAL EXUDATE AND OUTCOME OF DISEASE.

Culture of pleural exudate.	SSS in pleural exudate.	Cases.	Deaths.
Positive	Positive	16	5
		7*	3*
		2†	2†
Negative	Positive	3	1
		2*	0*
Negative	Negative	8	2
		1*	1*
		1‡	0‡
Total		27	8 (29.7%)
		10*	4*
		2†	2†
		1‡	0‡

* Positive blood culture without SSS.
† Positive SSS in blood without bacteremia.
‡ Positive blood culture and positive SSS in blood.

TABLE 3.—RELATIONSHIP BETWEEN DETERMINATION OF SSS IN PLEURAL EXUDATE, URINE AND BLOOD.

	SSS in pleural exudate.		
	Positive.	Negative.	Total
Total.	7	2	9
Positive SSS in urine Negative SSS in blood	3	0	3
Positive SSS in urine Positive SSS in blood	1	1	2
Total cases Positive SSS in urine	4	1	5
Total cases Positive SSS in blood	1	1	2

Pooled serum of Groups A to F, as used for the type identification of pneumococcal SSS in urine,[8] was successfully employed to determine the pneumococcus type involved in four experiments on pneumococcal pleural exudates. Four sterile pleural fluids obtained from tuberculous patients and used as controls did not contain detectable SSS when tested with the six different pooled sera.

The optimal concentrations of antibody (in mouse protective units) required for the detection of SSS in pleural exudates are apparently identical with those employed for the detection of SSS in urine, broth and plasma. Inhibition zones observed with other media occurred also with pleural exudates.

Two experiments were performed with SSS added to pleural exudates which contained no capsular polysaccharide. The SSS of pneumococcus Types I and III were detected in dilutions up to 1:800,000. The pH of the pleural fluids employed for these studies ranged between 7 and 7.5 (determined with "Nitrazine Paper," Squibb). These values are similar to those observed in experimentally produced pleural effusions of dogs and rabbits (Kelley, Scadron and Shinners[14]).

Summary. 1. Forty-four specimens of pleural exudate from 27 patients suffering from lobar pneumonia were examined bacteriologically and immunologically.

2. Twenty-four pleural exudates from 16 patients were positive on culture, and contained detectable capsular polysaccharide, but no antibody. Capsular polysaccharide was found in 6 sterile pleural fluids from 4 patients. On one occasion the pleural culture was positive. Fourteen fluids from 9 patients were negative on culture, and contained no SSS; in 8 of these fluids (from 4 patients) antibodies could be detected.

3. Eight of the 27 patients died (29.7%). Capsular polysaccharide was detectable in the pleural exudates of 6.

4. Thoracotomy after thoracentesis was performed in 11 cases (3 deaths) while thoracenteses alone were employed for the remaining 16 patients (5 deaths). More recoveries occurred in thoracotomized patients who had had bacteremia than in those given repeated thoracenteses.

5. In 9 patients where blood, pleural exudate, and urine were studied for capsular polysaccharide it was detected in 7 pleural exudates, in 5 urines after concentration, and in the 2 bloods.

6. Pooled serum was satisfactorily used for the detection of SSS in pleural exudates.

7. For the detection of capsular polysaccharide in pleural exudates the optimal concentrations of antibody are essentially the same as those used for capsular polysaccharide detection in urine, broth and plasma.

Miss Constance Lebair assisted in the statistical analysis and in the preparation of the paper.

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A METHOD OF DETERMINING THE PROBABILITY OF CONSTITUTIONAL REACTIONS DURING TREATMENT OF THE RAGWEED HAY FEVER PATIENT.*

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The occurrence of general or constitutional reactions after injections of pollen extract is one of the chief difficulties in the treatment of hay fever. Some of these reactions may be attributed to errors in technique or in judgment of dosage, but even the most experienced cannot avoid them entirely. Different patients suffering from the same type of hay fever vary so greatly in their response to treatment that the schedule of doses which is best for one may be entirely inapplicable to another.

Cooke[1,4] has devised a method of grading the degree of sensitivity of hay fever patients in three classes on the basis of the reaction to intracutaneous tests with pollen extract of various strengths, and has planned dosage schedules for each class. This system proved helpful in avoiding reactions from the first few injections of the series, but, as was recognized by its originator and will be shown subsequently, was unreliable in predicting the further course of treatment in any particular patient.

The present paper describes the use in skin tests of an antigen derived from ragweed pollen, which gives, much better than the regular pollen extract, an index of the ability of the ragweed hay fever patient to develop tolerance for injection of increasing doses of ragweed pollen extract, and of the probability of constitutional reactions to injections.

Chemical studies have shown that there are two main distinct antigenic fractions in ragweed pollen extract which are separated by fractional precipitation with ammonium sulphate at pH 4.[7] Practically all ragweed hay fever patients tested were sensitive to Fraction 1, some were equally sensitive to Fraction 2, while others gave little or no reaction to tests with this fraction in the dilutions ordinarily used for intracutaneous tests. When treated with regu-

* Presented before the meeting of The Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 3, 1941.

lar extract, which contains both fractions, almost all patients soon developed a blocking antibody[3,5] which inactivated Fraction 1, but only 1 in 13 developed antibody which inactivated Fraction 2.[6]

It seemed that if the blocking antibody had any real importance in the body, the patients who were sensitive only to Fraction 1 and developed antibody which inactivated it should do better under treatment than those who were sensitive to both fractions but developed blocking antibody inactivating only one. When a group of 13 cases was examined from this standpoint, there was no definite difference in the clinical protection against hay fever, but there was a striking difference in their tolerance for large doses of pollen extract. The patients reacting only to Fraction 1, in the dilution tested, took doses of 1500 to 10,000 protein nitrogen units (average 5300) without constitutional reactions; those sensitive to both fractions, who developed blocking antibody only for Fraction 1, reached doses of only 700 to 2000 (average 1360), but 4 of 6 had one or more constitutional reactions. On the other hand, the one case who was sensitive to both fractions and had blocking antibody for both took doses up to 7500 without any reaction.[6]

These results suggested that the blocking antibody was a factor in the development of tolerance for antigen injections and that, indirectly, sensitivity to Fraction 2 led to a higher incidence of constitutional reactions. Therefore skin tests with the two separate fractions might give an indication of cases which were particularly apt to have untoward reactions.

Two hundred and twenty-one ragweed hay fever patients were tested intracutaneously with suitable dilutions (containing 1, 10 and 100 protein nitrogen[2] units per cc.) of the two ragweed fractions prepared according to the method of Stull, Sherman and Hampton.[7] Many of these were tested because they had histories of repeated constitutional reactions, but others had taken large doses well, and still others were tested with the fractions before treatment was started, but have since received injections through one season. When the figures for all cases tested were summarized, it was found that about one-half of the patients in the whole group had one or more general reactions. Needless to say, this was considerably higher than the incidence in the clinic as a whole, due to the inclusion of so many "problem cases."

TABLE 1.—REACTIVITY OF RAGWEED-SENSITIVE PATIENTS TO FRACTIONS 1 AND 2 IN RELATION TO INCIDENCE OF CONSTITUTIONAL REACTIONS.

Intracutaneous tests.	Total cases.	Cases having constitutional reactions.	Percentage of cases having constitutional reactions.
F1 > F2	93	13	14
F1 = F2	108	81	75
F2 > F1	20	16	80

Table 1 shows the incidence of constitutional reactions in cases classified on the basis of the relative size of the skin reactions to the

two fractions. Ninety-three patients gave larger skin reactions to Fraction 1 than to Fraction 2. Of these, 13 patients (14%) had one or more constitutional reactions during treatment. Many of these were minor reactions which did not require administration of epinephrine or other drugs. However, since it is impossible to grade the severity of reactions accurately, all cases with any untoward symptoms attributable to the injections were grouped together, regardless of the severity or frequency of the reactions.

One hundred and eight patients had skin reactions of equal size to the two fractions. Of these, 81 (75%) had constitutional reactions. There were only 20 patients who showed larger skin reactions to Fraction 2 than to Fraction 1, but of these 16 (80%) had general reactions. Since the incidence of general reactions in the last two groups was more than five times that in the first group, it was apparent that these skin tests gave an index of the probability of a constitutional reaction.

However, both the theory outlined, and common sense, suggested that the actual degree of reaction to Fraction 2 was more important than the relative size of reactions to the two fractions. To compare the value of skin tests with Fraction 2 and those with regular ragweed extract as a basis for predicting constitutional reactions, we classified a group of 95 patients, A, B, and C, by the method of Cooke,[1] according as it required dilutions containing 10, 100 or 1000 protein nitrogen units per cc. of the regular ragweed extract to produce a *marked*[1] intracutaneous reaction with pseudopods. The same patients were then reclassified on a similar scale, using (for the tests) solutions of Fraction 2 containing the same amounts of protein nitrogen.

TABLE 2.—INCIDENCE OF CONSTITUTIONAL REACTIONS IN RAGWEED-SENSITIVE PATIENTS CLASSIFIED BY SKIN REACTION TO WHOLE RAGWEED EXTRACT AND TO FRACTION 2.

Class.	Total cases.	Cases having constitutional reactions.	Percentage of cases having constitutional reactions.
Classified by Test With Whole Extract.			
A	15	8	53
B	41	25	61
C	39	17	44
Classified by Test With Fraction 2.			
A	9	8	89
B	25	21	84
C	61	21	34

Table 2 shows the relative incidence of constitutional reactions in the three classes when the classification was based on tests with regular extract and when it was based on tests with Fraction 2. It was apparent that the reaction to regular extract was no criterion of the probability of general reactions. The moderately sensitive class (B) had a higher incidence of reactions than the exquisitely

sensitive class (A) and that of the least sensitive class (C) was only slightly lower. Reclassification on the basis of the reaction to Fraction 2 gave more information, Classes A and B having an incidence of reactions 2½ times that of Class C. One weakness of this method of dividing the classes was that almost two-thirds of all the cases tested fell into Class C.

TABLE 3.—SKIN REACTION TO FRACTION 2 (100 UNITS PER CC.) IN RELATION TO INCIDENCE OF CONSTITUTIONAL REACTIONS.

Intracutaneous tests.	Total cases.	Cases having constitutional reactions.	Percentage of cases having constitutional reactions.
Slight-negative	79	10	13
Moderate	64	34	53
Marked	71	62	87

Table 3 illustrates a classification which is fundamentally the same, but in which the groups are more nearly equal in size. Here 214 cases, including some not included in Table 2, are classified on the basis of the degree of reaction to test with a solution of Fraction 2 containing 100 protein nitrogen units per cc. Seventy-nine patients gave a negative or slight reaction; of these 10 (13%) had constitutional reactions. Sixty-four patients gave a moderate reaction, without pseudopods; of these, 34 (53%) had general reactions during treatment. Of the 71 persons who gave a marked skin reaction, 62 (87%) had constitutional reactions. In other words, the group most sensitive to Fraction 2 had 7 times as high an incidence of constitutional reactions as the least sensitive, while the group with moderate reactions was midway between. While these figures tell the story fairly well, they do not reveal one important aspect. Many of the patients in the less sensitive group who were listed as having constitutional reactions had one or two reactions, often following what in retrospect seemed an unwise increase in dosage, but subsequently went on and tolerated much larger amounts. On the other hand, practically all of the patients who reached a low "ceiling" dosage, above which they could never be raised without inducing a constitutional reaction, fell into the group most sensitive to Fraction 2.

Similar information was obtained when the reaction to the two fractions was tested by the method of passive transfer. In performing these tests, sites were made on the skin of the back of a nonallergic healthy test subject by injecting intracutaneously into each site 0.1 cc. of a 1 to 10 dilution of the patient's serum. These sites were tested 48 hours later with 0.025 cc. of solutions of the respective fractions, containing 1 protein nitrogen unit per cc. In a series of 89 cases (Table 4), the group whose sera sensitized normal skin to Fraction 2 gave an incidence of constitutional reactions almost 3 times that of the group whose sera did not react with this fraction in the dilution used. However, results obtained by this method gave no information not obtainable by direct skin test.

TABLE 4.—PASSIVE TRANSFER REACTION OF SERA (1 TO 10) TO FRACTION 2 (1 UNIT PER CC.) IN RELATION TO INCIDENCE OF CONSTITUTIONAL REACTIONS.

Passive transfer reaction.	Total cases.	Cases having constitutional reactions.	Percentage of cases having constitutional reactions.
0	34	11	32
±	9	5	56
+	20	17	85
++ or +++	26	22	85

No matter how the cases were classified, there were always a moderate number of exceptions. This could have been predicted from the serologic studies which showed that some patients (about 10%) do develop a blocking antibody for Fraction 2 in the early stages of treatment, while a few others develop blocking antibody for Fraction 1 either very slowly or not at all. No attempt was made to determine the blocking antibodies in the sera of most cases. However, serum was taken after treatment from 6 patients who were definitely sensitive to Fraction 2 but had taken large doses of extract without untoward reactions. Four of the 6 sera inactivated Fraction 2, showing that their exceptional behavior was due to the type of antibody present. Because of the technical limitations of the method used,[6] it was not possible to exclude the presence of small amounts of blocking antibody for Fraction 2 in the other two sera. On the whole, the results strongly suggested that the two fractions were specific antigens and that the blocking antibody was an important factor in acquired tolerance for injections of antigen.

More important, however, was the fact that intracutaneous tests with Fraction 2 seemed to offer a valuable criterion for judging the probability of constitutional reactions. The best basis for planning the range of doses of pollen extract for a ragweed hay fever patient is afforded by skin tests with whole extract and each fraction in dilution of 1, 10 and 100 protein nitrogen units per cc. The tests with the whole extract (and Fraction 1) give an indication, as shown by Cooke, of the safe initial dosage, while the tests with Fraction 2 indicate the probability of developing tolerance for increasing doses.

Many of the patients who gave strong reactions to Fraction 2 and were unable to take large doses of pollen extract, had excellent clinical results when treated with small doses of the regular extract. In some cases, where it was impossible to give enough of the regular extract to protect the patient from hay fever, treatment has been carried out with a solution of Fraction 1 alone. In a few cases, solutions of the two fractions have been injected separately, the dosage of each being increased according to the amount of local reaction to it. Although a few general reactions occurred, these trials suggested that many of these patients tolerate Fraction 1 better than the whole extract. However, the number of cases thus treated so far has not been large enough for final conclusions as to the degree of clinical protection.

Summary. Ragweed hay fever patients who are very sensitive to Fraction 2 of the pollen extract are less apt to develop tolerance for large doses of pollen extract than those who are only slightly sensitive to this fraction.

Intracutaneous tests with Fraction 2 help determine in advance which cases are more apt to have constitutional reactions and should be treated with special caution.

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STUDIES OF THE B VITAMINS IN THE HUMAN SUBJECT.

IV. MENTAL CHANGES IN EXPERIMENTAL DEFICIENCY.

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IN the course of a series of experimental studies of a deficiency of the B vitamins in man[6–9,15] we have observed that the deficient person seems confused and apparently is less able to reason and to exhibit good judgment than under normal conditions. Experiments in the rat[1–3,11,12,17a,b,18a–c,19–22,24–26] have shown impaired ability to learn a maze during deficiency of the B vitamins, but in no instance have accurate psychological measurements of mental changes in such a deficiency been attempted in the adult human being. Accordingly the present investigation* was undertaken to measure objectively the psychological status of the adult human subject taking an experimental diet deficient in the B vitamins and to demonstrate any changes which might occur following therapy with thiamine, brewer's yeast or the synthetic fractions of the B complex.

* We are indebted to Dr. Miles Murphy of the Psychological Clinic of the University of Pennsylvania for his coöperation.

Methods of Study. Psychological tests, grouped together as described below, were administered to 4 voluntary subjects taking an experimental diet and to 4 normal controls who consumed an adequate diet. The subjects, middle-aged women without complicating disease, were maintained under constant environmental conditions in the Thompson Vitamin Ward of the Philadelphia General Hospital. Each subject consumed a constant daily quantity of an experimental diet as described elsewhere,[3] which supplied an adequate quantity of all dietary factors* except the B vitamins. These were present in approximately one-half the theoretically required amount† for 3 of the subjects (D. C., E. V., R. A.) and were just short of the required amount for 1 subject (I. C.). The controls, women similar to the subjects in age, general intelligence and socio-economic level, were all trusted employees of the hospital. During this study they continued their usual activities and consumed without change the same adequate hospital diet to which they had been accustomed for many years.

The study was divided into three experimental periods: Period I, when the subjects received the experimental diet alone, during which time all developed the characteristic clinical manifestations of deficiency as described elsewhere;[7] Period II, when thiamine‡ was added to the diet and all improved, and finally, Period III, when the B complex was administered either as brewer's yeast§ or the synthetic B complex** and further clinical improvement occurred. Psychological tests were administered to the subjects at the end of each experimental period and to the controls at similar times.

Psychological Procedures. Psychological tests were selected which would measure as reliably as possible those functions which appear to be impaired in human beings and rats suffering from a deficiency of the B vitamins. The mental functions studied and the psychological tests employed are shown in Table 1. Details of the testing material used are given below. With the exception of a newly devised test of prose memory and a new arrangement of the Healy-Bronner Picture Memory Test, both of which are now being standardized, all are accepted psychological tests, the limits of error of which have been carefully worked out. Alternative forms of each test, equal in difficulty, permitted repeated testing of the same mental functions without the observations being influenced by practice effects. The different forms of each test were rotated so that no one form was used for all subjects in any experimental period.

Each subject and each control were subjected to four different psychological Test Series: both Test Series A and B, given 24 hours apart, were administered at the end of Period I (Deficiency), Test Series C at the end of Period II (Thiamine) and Test Series D at the end of Period III (B complex). Each series consisted of from five to eight different psychological tests†† and, with the exception of Test Series A, each consisted of a 2-day schedule, on consecutive days as on page 390.

* Cod-liver oil concentrate capsules, used as supplement to the diet, were kindly supplied by the White Laboratories, Inc., Newark, N. J., and the ascorbic acid was supplied by E. R. Squibb & Sons, New York City.

† The theoretical requirement for thiamine was calculated for each subject according to the Cowgill formula.[5] The thiamine content of the diet was calculated from the Cowgill tables and these values corroborated by chemical analysis on several occasions.[9] It was assumed that other B vitamins were present in analogous quantities.

‡ The thiamine used in the studies was generously supplied as "Betabion" by Merck & Co., Rahway, N. J.

§ We are indebted to Burroughs Wellcome & Co., New York City, for supplies of "Tabloid" yeast concentrate.

** Generously supplied as "Pentaplex" by Smith, Kline & French Laboratories, Philadelphia, Pa.

†† Alternative forms of each psychological test were used in the different Test Series.

TABLE 1.—SPECIAL FUNCTIONS STUDIED AND PSYCHOLOGICAL TESTS EMPLOYED

Mental functions* studied.	Psychological test employed.
Speed of hand muscle coördination	Whipple Healy Tapping Test
Intelligence	Henmon-Nelson Test of Mental Ability, High School Examination Forms A, B and C (for a preliminary test, Otis Quick Scoring Mental Ability Test, Beta, Forms A and B)
Reasoning ability (reading)	Thorndike-McCall Reading Scale, Forms 1, 2, 3, 4, and 5
Foresight and judgment	Porteus Maze Test, up and inverted
Prose memory	Newly devised test (results to be reported later)
Visual and auditory memory	Healy-Bronner Picture Memory Test (results to be reported later)

Test Series A.

1. Whipple-Healy Tapping Test (3 repetitions).†
2. Otis Quick Scoring Mental Ability Test.‡
3. Porteus Maze Test.

Test Series B, C and D.

First Day.

1. Henmon-Nelson Test of Mental Ability.
2. Whipple-Healy Tapping Test.
3. Prose Memory Test.
4. Picture Memory Test—A and B.

Second Day.

1. Porteus Maze Test.
2. Prose recall.
3. Picture recall.
4. Thorndike-McCall Reading Scale.

EXPLANATION OF PSYCHOLOGICAL TESTS. 1. *Porteus Maze Test.*[23] This test presents the subject with 10 increasingly difficult mazes in which he is to trace the correct path with a pencil (Fig. 1). The mazes measure aspects of foresight and judgment since the ability to solve such a problem is determined by the subject's capacity to look ahead and to weigh different factors which lead to a decision involving action. The subject's score is in terms of the number and difficulty of the mazes with which he succeeds.

In order to have an alternative series of tests equal in difficulty, the mazes were used inverted as well as in the standard position. This arrangement was suggested by Dr. Porteus on the basis of data in his possession. A "point score" rather than the usual "age score" was employed, again at Dr. Porteus' suggestion. The mazes so used are apparently of equal difficulty, the average score for all our subjects and controls with the maze in the upright position being 68.6 and with the maze in the inverted position, 69.3 (not a significant difference). In computing results, comparison was made only between tests in which the mazes were presented in the same position.

* The statement of mental functions given here is for the purpose of clarifying the experiment for readers who are not psychologists; it is not intended to be a technical delimitation of mental "factors," "primary mental abilities" or the like.

† Some improvement in performance on this test is obtained through initial experience. For this reason it was given three times here to allow for initial improvement.

‡ Published by World Book Company, Yonkers-on-Hudson, New York City, 1937. Forms Beta A and B were used. This test was used as a preliminary exploration of the intelligence of the subjects and controls; the results were not used further in the study.

2. *Henmon-Nelson Test of Mental Ability.** This is a standardized intelligence test measuring general competency of thinking. Forms A, B and C, High School Examination, were utilized in this study. The subject's task is to follow out a variety of different directions. The original score is in points which may be translated either into terms of mental age or rate of mental growth (Intelligence Quotient).

3. *The Thorndike-McCall Reading Scale.*† Forms 1, 2, 3, 4 and 5. This test consists of a series of paragraphs of increasing difficulty which must be read by the subject and to each of which is attached a series of standardized questions on the content of the paragraph. The test measures the ability to read comprehendingly and may therefore be regarded as fundamentally a measure of the reasoning process.

The subject's score is in terms of the number of questions correctly answered. A translation table allows the score also to be read in terms of the elementary or high school grade reading score, in terms of reading age or in terms of a standard score called "T" score.

4. *Whipple-Healy Tapping Test.*[4] This test presents the subject with a page of ½-inch squares, 10 in a row and 15 in a column. The subject's task is to place a dot with a pencil in each square across one row and back on the next, and so on, without touching any lines, making as many taps as possible in 30 seconds. Two trials are given at each testing period and the average of the two trials is the score used. The score is the number of taps made minus twice the number of errors. The speed of tapping is a highly stable phenomenon and is only temporarily subject to practice effects.[13]

5. *Test of Memorization of Short Prose Passages.* We have devised six alternative prose passages used as a test of memorization. Data from comparable age, socio-economic and intelligence groups necessary for standardization of these tests is now being accumulated and results will be reported later.

6. *Healy-Bronner Picture Memory Test.*[4] In this test the subject views a picture while the experimenter reads to him certain relevant material. His task is to repeat as much as possible of the content of what was read while still looking at the picture. This procedure constitutes a measure of combined visual and auditory memory. Further data are required to standardize the test in this form and results will be reported later.

Statistical Procedures. Because the number of subjects was necessarily limited it was essential to employ extensively the statistics of small samples[10,16] in order to arrive at a satisfactory interpretation of the results. The statistical procedures utilized will be given briefly in the present report, and a more detailed description both of the statistical handling of the data and of the procedures followed in the giving and scoring of the tests, will be published elsewhere.

Clinical Status of the Subjects. All subjects showed definite clinical evidence of deficiency when Test Series A and B were administered; had improved, following therapy with thiamine, when Test Series C was given, and remained improved, following therapy with brewer's yeast or with the synthetic B complex, when Test Series D was given (Table 2). The degree of deficiency, as judged by the number and severity of the clinical manifestations, varied from mild to moderately severe, depending on the amount of thiamine and other B vitamins which each subject received in her diet and on the time during which the deficient diet was consumed. Thiamine was administered in varied dosage to the different subjects so that the length of time required to relieve manifestations of deficiency differed, and, therefore, the intervals between the different psychological Test Series varied

* Published by the Houghton Mifflin Company, New York City, 1935.

† Published by the Bureau of Publications, Teachers College, Columbia University, New York City, 1931.

for the different subjects. Therapeutic effect from brewer's yeast and from the synthetic B complex appeared to be comparable for the relatively short period during which these observations were made.* However, certain differences between the two have been noted when clinical observation is prolonged beyond the limits of this study.†

TABLE 2.—CLINICAL MANIFESTATIONS IN THE 4 EXPERIMENTAL SUBJECTS DURING DEFICIENCY (PERIOD I); AFTER THE ADMINISTRATION OF THIAMINE (PERIOD II); AND OF THE B COMPLEX (PERIOD III).

Subject.	Age.	Daily intake of thiamine.	Average daily excretion of thiamine during Period I.* μg.	Clinical Manifestations.		
				Period I. (Deficiency.)	Period II. (Thiamine added.)	Period III. (Brewer's yeast or synthetic B complex added.†)
D. C.	65	205	33	(AFTER ? DAYS)‡ Easily fatigued, anorexia; malnutrition; smooth red tongue; patellar reflex hypoactive; sense of vibration lost and muscle tenderness present in legs.	(AFTER 4 DAYS) Strength and appetite improved; incr. of 2 pounds in body weight; tongue unchanged; patellar reflex more active; vibration perceived over malleoli; muscle tenderness decr.; sl. edema.	(AFTER 31 DAYS) Strength and appetite normal; further incr. of 7 pounds in body weight; tongue less red; patellar reflexes normal; vibration perceived imperfectly in both legs; no edema.
E. V.	48	204	21	(AFTER 62 DAYS) Anorexia; epigastric discomfort; palpitation; neuritic pains in face; red tongue; sl. muscle tenderness; activity reduced.	(AFTER 10 DAYS) Anorexia diminished; epigastric discomfort unchanged; no palpitation; no neuritic pains; red tongue; muscle tenderness decr.; activity incr.	(AFTER 10 DAYS) Appetite normal; epigastric discomfort improved; incr. of 2 pounds in body weight; tongue normal; muscle tenderness decr.; activity normal.
R. A.	43	353	35	(AFTER 74 DAYS) Easily fatigued; anorexia, marked epigastric discomfort and nausea; nervousness, irritability, apprehension; red tongue; marked muscle tenderness; mod. edema.	(AFTER 8 DAYS) Strength and appetite impr.; no other change.	(AFTER 15 DAYS) Strength normal; sl. anorexia and epigastric discomfort; no nausea; no nervous manifest.; tongue normal; no edema or muscle tenderness.
I. C.	65	574	42	(AFTER 98 DAYS) Marked weakness, anorexia and epigastric discomfort; occ. vomiting; shifting abd. pain; dysp. on exertion and palpitation; abd. pain; paresthesias and pain in legs; sense of vibration impaired; marked muscle tenderness; sl. edema.	(AFTER 38 DAYS) Strength mod.; appetite fair; epigastric discomfort mod.; no abd. pain or vomiting; no cardiac symptoms; no pains or paresthesias; sense of vibration normal; mod. muscle tenderness; edema incr.; dry skin.	(AFTER 11 DAYS) Strength unchanged; appetite normal; sl. epigastric discomfort; paresthesias again marked; vibration normal; muscle tenderness incr.; edema reduced; skin impr.

* Twenty-four-hour urinary excretion of thiamine was determined daily according to a modification of the thiochrome method as described elsewhere.[9]

† Subjects D. C. and E. V. received synthetic B complex; Subjects R. A. and I. C., brewer's yeast.

‡ This subject was deficient on admission at which time Test Series A was given. She was immediately placed upon the experimental diet which was continued throughout observation.

Figures in parentheses indicate the duration of each experimental period prior to obtaining the clinical manifestations recorded, at which times the different psychological Test Series were given.

Evaluation of deficiency at end of Period I: D. C., mild; E. V., mild; R. A., moderately severe; I. C., moderately severe.

* The term "B complex" will be used in the text to denote either brewer's yeast or the synthetic B complex.

† Unpublished observations.

Results. 1. *Foresight and Judgment as Measured by the Mazes.* The subjects succeeded less well in solving the mazes (Fig. 1) when deficient than after they had received thiamine or the B complex. The mean scores for the group (Table 3) when deficient were 40 and 54.5 (Test Series A and B), as compared with 78.87 and 81.75 following administration of thiamine or of the B complex respectively (Test Series C and D). When deficient the subjects' ability to solve the mazes was inferior to that of the controls, but following therapy with either thiamine or the B complex their performance was equal to or better than that of the controls (Table 4). The difference between deficiency and recovery in each subject and the difference in the average for all subjects in deficiency and recovery

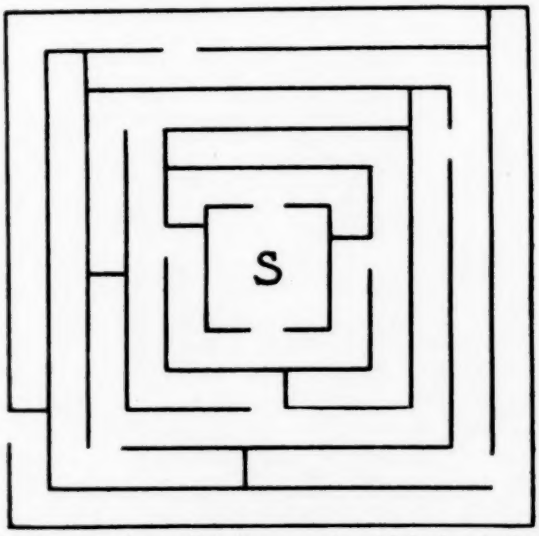

FIG. 1.—A maze of moderate difficulty occupying eighth place in the series of 10. For a successful solution, the subject having placed his pencil at "S" must draw a continuous line from this point to the exit without crossing any lines, and without retracing his path at any point. (Reproduced by courtesy of Dr. S. D. Porteus, Univ. of Honolulu).

were statistically significant (Table 3). Between the subjects when deficient and the controls at the same period the difference was close to statistical significance (Table 4), suggesting that with a larger number of cases the statistical significance would be established. Furthermore, a definite relationship existed between the degree of deficiency and the degree of impairment of maze performance.* The performance of the controls, on the other hand, showed no statistically significant variation throughout observation.

* The statistical correlation between degree of deficiency and actual raw score on the mazes was determined in various ways, and the results suggest that the individual's initial score on the mazes could be fairly well foretold on the basis of his degree of deficiency.

TABLE 3.—MAZE SCORES* OF SUBJECTS IN THE DIFFERENT EXPERIMENTAL PERIODS, AND OF CONTROLS TESTED AT THE SAME TIME INTERVALS.

SUBJECTS.

Period.	Test Series.	Initials. I. C.		D. C.		E. V.		R. A.		Mean, all subjects.	Changes from deficiency to therapy (thiamine of B complex). Test Series A to Test Series C.	Test Series B to Test Series D.	Series (A + B) to Series (C + D).	Significance of changes. P‡ (.05 and less are significant).
Maze position →		Up.	Inverted.	Up.	Inverted.	Up.	Inverted.	Up.	Inverted.					
I (deficiency)	A	74.5		31		32		22.5		40.0	+38.87			.032
	B		55.5		41		42.5		79	54.5				
II (thiamine)	C	61.5		90.5		72.5		91		78.87		+27.25		.022
III (B complex)	D		92		68		75.5		91.5	81.75			+33.06	<.01

CONTROLS.†

		M. Hl.		M. Hd.		M. F.		M. Hw.		Mean, all controls.				
In all Test Series the controls were tested on approximately the same day as the subjects	A	56		65.5		51		90.5		65.8	+5.12			.75
	B		67.5		77		56		93	73.4				
	C	47.5		51		87		98		70.92		+10.00		.36
	D		89.5		72.5		73.5		98	83.4			+7.56	.54

* Analysis of performance on the 3 most difficult mazes showed that deterioration associated with clinical deficiency occurred not only in the most difficult mazes but throughout the whole set of 10 mazes. Therefore only total scores for each entire set are here recorded. A perfect score would be 98.

† Maze score is known to rise upon retesting. Rise in score of the subjects must therefore be evaluated in relation to small increases in score obtained by the controls.

‡ P = Significance of the difference, or the probability that the observed difference might have occurred by chance. When P is 1.00 the difference is a purely chance affair. If P is .05 there are only 5 chances out of 100 that the difference could have occurred by chance. As the value of P decreases the likelihood increases that a difference in the observed direction will be consistently found.

These results indicate that deficiency of the B vitamins is associated with impaired ability to solve a series of mazes, that this impairment begins early in the deficiency and progresses as the deficiency deepens and that it may be restored by the administration of either thiamine alone or the B complex. From these facts it might be inferred that thiamine was the active factor in the results obtained and that other members of the B complex produced no further effect.* Before such an inference is acceptable observations should be made upon subjects receiving the entire complex over a prolonged period to determine whether any further improvement in maze score is possible.

TABLE 4.—AMOUNT BY WHICH THE MAZE SCORES OF THE SUBJECTS IN THE DIFFERENT EXPERIMENTAL PERIODS EXCEEDED OR FELL SHORT OF THE SCORES OF THE CONTROLS AT EACH PERIOD.

Period.	Test Series.	Differences. Mean of subjects minus mean of controls.	Significance of differences. P* (.05 and less are significant).
I (deficiency)	A	−25.75	.14
	B	−18.87	.16
II (thiamine)	C	+ 8.00	.61
III (B complex)	D	− 1.62	.86
Test Series (A + B) (deficiency)		−22.31	.087†
Test Series (C + D) (therapy)		+ 3.14	.73

* See Table 3 for definition of P.

† P = .087 is calculated from the average score for Test Series A and B for each subject and control. If the difference between subjects and controls is treated on the assumption that A and B are independent tests, then P = .03.

In view of the consistent and statistically significant improvement in each subject's performance on the maze after therapy as compared with her performance when deficient and in view of the essentially constant performance of the controls over a similar period of time, it seems clear that in this group of subjects, in whom the other experimental factors were constant, the changes observed were due to the therapy employed. These observations should in no sense be construed as implying that a vitamin B complex deficiency is the only factor which may result in poor maze performance. The results may be accepted, however, as indicating that an impaired maze performance is a significant manifestation of deficiency of the B vitamins.

Morbidity alone presumably does not affect maze performance adversely. At least data obtained in studies on rats[1,11,24] show that inanition does not reduce performance whereas a specific deprivation of the B vitamins does do so. Further elucidation of this problem in the human, however, is necessary.

It appears to be well established that in order to solve a maze

* Because of the necessity of using the mazes in different positions it was not possible to compare directly the effects of thiamine and the B complex, but the improvement achieved from deficiency to the thiamine period (Test Series A to Test Series C) was analogous to that achieved from deficiency to the B complex period (Test Series B to Test Series D).

successfully one must be capable of considering a number of factors simultaneously and of choosing between the alternatives on which ultimate success depends. The exact extent to which the findings on the maze may be applied to problems of everyday life is still not certain, but one may assume that when in daily life a person deficient in the B vitamins must exercise the mental functions that are required to solve a maze, namely, foresight and judgment, he will do so less adequately than the person properly supplied with these factors.

2. *Intelligence Tests.* There was no significant difference in initial intelligence between the subjects and the controls, both being in the dull normal group of adult intelligence. Averaging the results of all the Tests, the intelligence quotient of the subjects was 85.7 (mental age 13 years and 9 months) and of the controls, 78.7 (mental age 12 years and 8 months).

No significant differences were observed in intelligence test scores during deficiency or after therapy. The controls likewise showed no significant differences from one testing period to another. These results indicate that adult intelligence test performance does not deteriorate during deficiency of the B vitamins of the degree here studied nor does it improve after therapy with these substances.

3. *Reasoning Ability* (*Reading*). The Thorndike McCall reading tests indicated that the subjects and controls read with a comprehension to be expected for their mental age levels. The difference between the subjects and the controls on reading score was not a significant difference. A summary of all testing periods showed the following performance:

	School Grade in reading.	Reading Age.	T* Score.
Subjects	7.3	13 yrs. 7 mos.	55.7
Controls	6.1	12 yrs. 4 mos.	48.6

* T score is a standardized score more suitable for statistical procedures than other translations of the score.

No significant changes in the subjects or in the controls were noted from any testing period to any other testing period.

4. *Tapping Performance.* Although the controls at each testing period were somewhat faster than the subjects the differences between them were within the range to be expected by chance. An average of all performances at the three testing periods for the subjects and for the controls showed the following number of taps in 30 seconds: Subjects, 74.2; Controls, 82.7.

In the case of tapping, as in that of reading comprehension and in general intelligence vitamin B deficiency apparently did not cause deterioration, nor vitamin B therapy improve tapping speed and accuracy.

Conclusions. From observations on 4 adult subjects and 4 controls we may conclude:

1. Foresight and judgment, as measured by performance on maze

tests, are impaired when the subjects are deficient in the B vitamins and are improved after therapy with thiamine or with the B complex.

2. General intelligence, reasoning ability (reading) and speed of hand muscle coördination (tapping) show no measurable deterioration when the subjects are deficient in the B vitamins and no improvement after therapy with thiamine or with the B complex.

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CHANCRE OF THE GUM. A CASE REPORT.

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The present report of a case of chancre of the gum is prompted by: 1, the rarity of this lesion; 2, the unusual appearance of chancre of the gum when compared with primary syphilitic lesions at other sites;

and 3, the fact that the syphilitic nature of the lesion may not be readily recognized.

The rarity of chancre of the gum is indicated by the estimate[4,7] that this primary lesion accounts for only 0.46 to 1.4% of extragenital chancres, which, though not rare, are unusual, since they constitute only 8 to 9% of all chancres. Klauder,[4] in 1921, reporting a case of chancre of the gum, surveyed the world literature concerning this subject and found the number of cases recorded, including his own, to be 113. In 1937, Straith,[7] reported an additional case. He carefully combed the literature which had appeared subsequent to Klauder's report and discovered 42 additional cases. The only communication noted in the literature since then has been a single case report by Scarpa[6] which brings the total of cases reported to 157. Very few of these cases are in the American and English literature. Indeed, since Klauder's report in 1921, only 3 cases have been cited—Cabot[2] in 1930, Epstein[3] in 1933, and Straith[7] in 1937.

Case Report. The patient was a 19-year-old unmarried negress, a domestic, who was first seen in the Pediatric Out-patient Clinic of the New Haven Hospital at the age of 9. Following that observation, she appeared in the various Out-patient Clinics of the hospital from time to time for reasons that are irrelevant to this presentation. During this period, routine serological tests for syphilis were carried out on her blood with negative Kahn reactions being reported in 1930, 1934 and 1937.

On May 7, 1940, the patient appeared at the Medical Out-patient Clinic complaining of tenderness of the left upper gum. She reported that one week previously she had noticed a sore, red spot on the gum above the left lateral maxillary incisor. At first, it was no larger than the head of a pin, but over the period of a week it rapidly increased to almost the size of a dime. It became quite painful and bled easily when she brushed her teeth. Associated with this, a small painful lump appeared in the left side of her neck. Examination on May 7, 1940, revealed a reddish, soft, tender, raised area of gum about 1.0 cm. in diameter over the left lateral maxillary incisor and canine teeth. This lesion bled easily when touched. There was also noted an enlarged tender cervical node at the left angle of the jaw.

Following this examination she was referred to the Dental Clinic where, on May 15 and 16, 1940, dental prophylaxis was given and sodium perborate mouth washes were prescribed. Because of the unusual character of the lesion, she was returned to the Medical Clinic on May 23, 1940, complaining that the gum lesion was worse and that the lump in her neck had increased in size and had become more painful. Examination of the gum lesion on this date was as follows: The lesion was on the labial gingiva overlying the alveolar bone covering the roots of the two left maxillary incisors as well as portions of the left cuspid and right first incisor extending down over portions of the crowns of these teeth. It was about 1 cm. by 2 cm. in diameter and was uniformly elevated about 3 mm. throughout, with a clearly defined border. In contrast to the normal, firm, pink surrounding gingiva this lesion was non-pigmented, spongy, and had a bright deep red color. The dark cloudy areas scattered over the normal gingivæ which are shown in the illustration are, of course, normal pigmentation common in the colored race. The surface of the lesion glistened and was somewhat granular and cauliflower-like in appearance. There was some

bleeding but this was not excessive even on manipulation. Although the patient was quite apprehensive during the examination, the pain which she experienced when the lesion was touched or manipulated was probably quite genuine. There was regional lymph node involvement associated with the lesion, the submaxillary and anterior cervical lymph nodes being hard, moderately enlarged, and quite tender. Motion of the jaw was limited. Roentgen ray examination of the teeth under the lesion revealed no abnormalities. A Kodachrome color plate of the lesion is shown in Figure 1.

For the first time, the possibility of a chancre was considered. Serological examination of the blood on this date revealed a 4+ Kahn reaction, a 4+ Wassermann reaction with the cholesterolized antigen, and a negative Wassermann reaction with the alcoholic antigen. Repeated serological studies of the blood demonstrated a 2+ Wassermann reaction with the alcoholic antigen on May 28 and a 4+ Wassermann reaction with the alcoholic antigen on June 4. Darkfield examination of the gum lesion on May 25 was negative for *Tr. pallidum*.

Close examination of the personal history of the patient now revealed the following significant information. A 30-year-old negro, with whom the patient had been having sexual intercourse for 5 months, was examined on June 4, 1940. He was found to have mucous patches (dark field positive) in both tonsillar fossæ and on the inner surface of the lower lip. His blood revealed a 4+ Kahn reaction and a 4+ Wassermann reaction with both alcoholic and cholesterolized antigens. At the same time a 20-year-old sister of the patient, who also had engaged in sexual intercourse with the same man, was found to have mucous patches (dark field positive) in her mouth and a 4+ Kahn reaction and a 4+ Wassermann reaction with both antigens. The patient steadfastly denied acts of sexual perversion.

On June 6, 1940, following an intramuscular injection of 0.2 gm. of bismuth subsalicylate in oil, she was admitted to the Medical Service of the New Haven Hospital for antisyphilitic therapy.

Examination revealed the following significant changes: The chancre was somewhat diminished in size and in elevation and did not bleed on manipulation. It was only slightly tender to touch. In addition to the cervical adenopathy, there was a slight enlargement of the axillary and epitrochlear lymph nodes. There were no genital lesions.

The following laboratory data preparatory to therapy were secured:

Blood: R.B.C. 4,160,000, Hb. 75% (14.5 gm. = 100%), platelets, 250,000; W.B.C. 9,000, Diff. 70% seg. polymorph. neutrophils, 5% non-seg. polymorph. neutrophils; 17% lymph., 7% monos., 1% eosinophils.

Icteric Index: 6.

Urine: Clear, straw, acid reaction, sp. gr. 1.020; al. and sug. negative; centrifuged sed. negative for R.B.C., W.B.C. or casts.

Stool: Soft, formed, dark brown. Guaiac, negative. *Phenolsulfonphthalein urinary excretion:* 65% in 2 hours. *Liver function test:* No retention of bromsulphalein after 30 min.

Roentgen ray of Chest: Negative for pulmonary or cardiac lesions.

Since January, 1940, the 5-day massive dose arsenotherapy[1] of syphilis by the intravenous drip method has been employed at the New Haven Hospital[5] for the treatment of primary and secondary syphilis. Accordingly, this method of therapy, with the administration of a daily dose of 240 mg. of Mapharsen (arsenoxide), or a total of 1200 mg. of the drug in 5 days, was carried out on the patient from July 8 to July 12, 1940. During this period there was a slight but definite response of the chancre in that the degree of elevation over the surrounding gum was diminished, and the granular and cauliflower appearance noted heretofore changed to that of a normal smooth surface. Following this initial response the only change

noted in the lesion was the substitution of a more normal pink appearance in place of the bright red color. There still remains (March 18, 1941) a slight thickening of the gum at the site of the lesion, probably due to fibrosis and scarring.

The course of the serological changes are detailed in Table 1. It is apparent that the first serological change occurred 4 weeks after therapy and that complete seroreversal was obtained by 19 weeks' therapy.

TABLE 1.—SEROLOGIC FINDINGS.

	Serology.				
		Wassermann.			
Date.	Kahn.	Alc. antigen.	Chol. antigen.	Weeks after therapy.	Notes.
1930					
Mar. 14	−				
1934					
Sept. 12	−				
1937					
Mar. 17	−				
1940					
May 23	4+	−	4+		Picture of gum lesion taken
May 25	..	..	..	..	Darkfield examination of chancre negative
May 28	4+	2+	4+		
June 4	4+	4+	4+		
June 8–12	..	..	..	..	Massive arsenotherapy treatment
June 19	4+	4+	4+	1	
June 25	4+	4+	4+	2	
July 5	4+	3+	4+	3	
July 11	4+	2+	4+	4	
July 19	4+	−	4+	5	
July 23	4+	−	3+	6	
July 30	4+	−	3+	7	
Aug. 16	2+	−	−	9	
Sept. 19	2+	−	−	14	
Oct. 17	2+	−	4+	18	
Oct. 25	−	−	−	19	
Oct. 29	−	−	−	20	
Nov. 5	..	..	..	21	Lumbar puncture; Pandy neg., 1 lymphocyte per mm.^{3}, colloidal gold curve 0000000000, Wasserman negative.

Discussion. Klauder[4] has described in detail two types of chancre seen on the gum: the abrasive or erosive type, into which group the present reported case falls, and the ulcerative type. The former is the more easily recognized type, with a smooth glistening, convex, carmine-colored surface. The border is usually sharply demarcated from the surrounding tissue and the lesion may vary in size from a few millimeters to 2 or 3 cm. in diameter. Dark-field examination for *Tr. pallidum* is usually positive. The second, or ulcerative type, is more difficult to recognize and is described by Klauder as an ulceration of variable size and configuration with or without a sharply circumscribed edge.

FIG. 1.—Chancre of the gum, May 23, 1940.

These chancres are associated with a more or less painless adenopathy of the maxillary nodes. This occurs early and the nodes are frequently of cartilaginous hardness. It is noteworthy that the chancre occurs almost always on the upper anterior gum and with surprising frequency on the left side. Location of the lesion on the posterior aspect of the gum is seen with extreme rarity.

Differential diagnosis, if the possibility of gum chancre is kept in mind, is not ordinarily difficult. Darkfield examination, serological tests for syphilis, and the presence of a marked unilateral maxillary adenopathy, usually hard and non-tender, provide the basis for the correct diagnosis in the majority of cases. Interpretation of the darkfield may, of course, be difficult at times because of the presence of spirochetes in the mouth other than *Tr. pallidum*.

The lesion must ordinarily be differentiated from hypertrophic gingivitis and epulis. Chronic or subacute hypertrophic gingivitis is usually more generally distributed over all the gingivæ. It does not tend to localize or be circumscribed, nor is it so clearly demarcated from the surrounding normal structure. Hypertrophic gingivitis commonly originates and usually extends along the marginal gingivæ and is more ulcerated. The color is less bright red and more blue as in congestion and does not show the glistening granular and cauliflower reaction seen in chancre. It is not so painful to touch nor is the patient so apprehensive, as a rule. Regional lymph gland enlargement is not a common accompaniment of hypertrophic gingivitis.

The lesion shown here may conceivably be confused with certain kinds of epulides, especially the younger vascular type. These tumors, however, have a more rounded form, are not so uniformly elevated and show a marked tendency towards pedunculation. They may have a glistening deep bright red color as seen in this case, but often tend to bleed profusely owing to the vascularity commonly observed in the early stage. Again they are not so painful to touch on manipulation nor is regional lymph gland involvement associated with them.

The authors are indebted to Mr. Howard J. Reynolds, photographer, Yale University School of Medicine, for the photography of the colored illustration in this report.

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THE DURATION OF ACTION AND THE ACTIVITY OF DIFFERENT SIZE DOSES OF PROTAMINE ZINC INSULIN.

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The clinical literature gives no sharply defined statements on the comparative duration of action of different size doses of protamine zinc insulin. Opinions on the duration of action and time of maximum activity of a given dose vary from worker to worker. A few quotations will illustrate this diversity of opinion. Wilder[10] states: "The activity of protamine insulin is prolonged for more than 48 hours in fasting diabetic patients. The duration of action of protamine insulin when food was given exceeded 36 hours." Aitken[1] says: "Observations of the effect of protamine zinc insulin on the glycosuria of diabetics receiving constant diets (usually in 3-hourly feedings) indicate that a large and possibly maximal insulin effect is exerted on carbohydrate metabolism within 3–6 hours of the injection, and that the total duration of effects of doses lying between 15 and 100 units is 15–60 hours." Neuhoff and Rabinovitch,[8] however, state that the lowest blood sugar, if protamine zinc insulin is given at 7 A.M., will be obtained in nearly all cases after 20 hours. On the other hand, if the injection is given at 10 P.M. the low point will in most cases occur after 5 hours. Others, Mark[6] and Ricketts,[9] in contrast speak of the constancy of action of protamine zinc insulin. Kerr and Best[4] also conclude: "Up to a point the effect of increasing the dose of protamine zinc insulin is seen to be that of prolongation of its action rather than a further depression of the blood sugar." R. D. Lawrence[5] is one of the few who gives any quantitative statement regarding the duration of effect of different size doses and time of maximum effect. His table of action is as follows:

Protamine zinc insulin (units)	Duration (hrs.)	Commonest time of hypoglycemia (hrs.)
10	6–8	5
20	12	8–12
30	18–24	8–20
40	24	16–24

The studies to be presented in this paper are the attempt to clarify this problem of the duration of action and the activity of different size doses of protamine zinc insulin. We were unable completely to interpret our clinical results until we had studied the

problem in the depancreatized dog. These results, therefore, will be presented first.

The depancreatized dog was selected for the study of the duration of action of protamine zinc insulin for two reasons: 1, There is no pancreas present to confuse the issue by the secretion of an unknown amount of insulin. 2, There is a fasting insulin need, or basal insulin requirement[3] in these dogs. When the absorption of insulin, which has been injected, falls below the basal requirement the blood sugar rises abruptly. This gives a sharp end point for the measurable activity of injected insulin. This is a fact of real importance in determining the duration of action of protamine zinc insulin. Two depancreatized dogs were used which had a marked difference in their basal insulin requirements. This was done in order to determine the relationship of the basal insulin requirement to the duration of action of protamine zinc insulin. Dog D had a basal requirement of 0.3 unit per hour and Dog C, a low basal insulin requirement of 0.16 unit per hour.

The effects of single injections of 5, 10, 20 and 40 units of protamine zinc insulin were studied at approximately weekly intervals. The dogs were fasted for 12 hours prior to the tests and during the test. The insulin activity was measured by the amount of intravenous glucose required to maintain a normal blood-sugar level. The end of measurable activity was considered to occur when the blood sugar curve showed a rising level. (The dogs remained in good condition during the weeks of study and had no significant changes in diet or insulin requirement.) The results obtained are shown graphically in Charts 1 and 2, and are summarized in Table 1.

TABLE 1.—DURATION OF ACTION OF DIFFERENT SIZE DOSES OF PROTAMINE ZINC INSULIN IN DEPANCREATIZED DOGS.

Summary of Charts 1 and 2.

Size of dose of protamine zinc insulin (units).	Duration of measurable activity in hours.		Peak of action in hours.		Intravenous glucose required, gm.	
	Dog D.*	Dog C.†	Dog. D.	Dog C.	Dog D.	Dog C.
5	16	20	11–16	15–19		
10	22½	24	16–22	15–24	..	2.7‡
20	25	51	13–20	14–24	7‡	9‡
40	25	..	12–20	..	5‡	

* Basal insulin requirement Dog D, 0.3 units regular insulin per hour.

† Basal insulin requirement Dog C, 0.16 units regular insulin per hour.

‡ More glucose could have been given as the blood sugar was in the hypoglycemic range at several points.

1. *The Duration of Action of Different Size Doses in: (a) The Same Animal.* It is obvious from the charts that the duration of action varies with the size of the dose, but is not directly proportional to the size of the dose. This is clearly illustrated by Dog D, where 5 units lasted 16 hours, 10 units 22½ hours, 20 units 25 hours and 40 units 25 hours. The actual activity of both 20 and 40 units probably lasted longer than the 25 hours of measurable activity,

but was masked by the high basal insulin requirement of this dog. In other words, the insulin being absorbed was below the amount required to maintain the blood sugar level.

Diana: Different size doses in same animal to contrast activity and duration of action

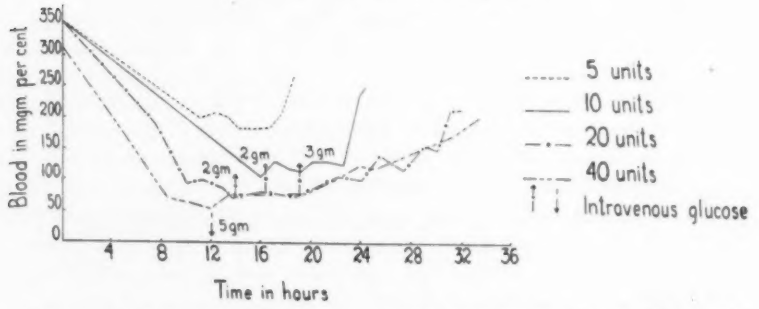

CHART 1.—Effects on the blood sugar of single injections of 5, 10, 20 and 40 units of protamine zinc insulin. The amount and time of injection of intravenous glucose is indicated by arrows. All the arrows pointing upward refer to the 20-unit curve, while the single downward arrow refers to the 40-unit curve. Basal insulin requirement 0.3 units regular insulin per hour.

Cricket: Different size doses in same animal to contrast activity and duration of action

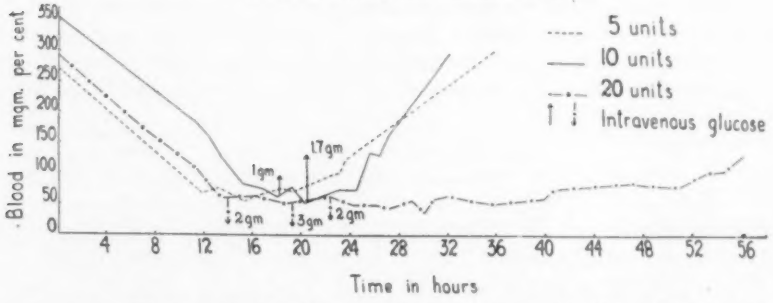

CHART 2.—Effects on the blood sugar of single injections of 5, 10 and 20 units of protamine zinc insulin. The amount and time of injection of intravenous glucose is indicated by arrows. All the arrows pointing upward refer to the 10-unit curve, while the arrows pointing downward refer to the 20-unit curve. Basal insulin requirement 0.16 unit regular insulin per hour.

(*b*) *Different Animals.* The difference in the duration of action of the same dose in different animals is striking. In every instance Dog C with a lower basal requirement showed a longer duration of action: 51 hours for 20 units as contrasted with 25 hours in Dog D (see Table 1).

2. *The Time Activity Function.* The gradient of fall of the blood sugar is only slightly more rapid with the larger size doses of protamine zinc insulin. This indicates that the rate of absorption is not

proportional to the size of the dose. This may be related to the fact that the surface area of the injected insulin is proportionally greater with smaller volumes. Thus in Dog D, 40 units caused a fall from 360 to 72 mg. per 100 cc. in 13½ hours and 5 units a fall from 300+ to 180 mg. per 100 cc. in 14 hours.

The time of the peak of activity appeared to vary only slightly with the size of the dose but the effect was greater with the larger size doses. This is demonstrated by comparing the activity of 5 units and 20 units in Dog D. Five units caused a moderate fall in the blood sugar level in the first 11 hours, a plateau for 5 hours and a sharply rising blood sugar level between the 18th and 17th hours. In the experiment with 20 units, 7 gm. of glucose were required between the 14th and 19th hours to prevent severe hypoglycemia. This was followed by a relatively constant blood sugar level until the 25th hour when the blood sugar slowly started to rise. The only slightly greater increase of effect of 40 units as compared with 20 units in this same dog illustrates the relative decrease in effectiveness per unit with increasing dosage, a fact well established for regular insulin.[2]

It should be stressed that the actual absorption of small amounts of protamine zinc insulin may last far longer than the measurable effect on the blood sugar, as explained above.

TABLE 2.—DURATION OF ACTION OF PROTAMINE ZINC INSULIN IN PATIENTS.*

Patient.	Sex.	Age.	PZI in units.	Duration of action in hrs.†	Peak of activity.	Basal rate per hr.	Glucose per os in gm.
1. F. B.	F	13	10	8	6+	1¼	0
2. L. S.	M	53	30	12+	?	?	38
3. A. C.	M	26	30	24+	? ?	0	175
4. M. F.	F	45	30	24+	18–24 ?	?	0
5. J. S.	M	16	40	18	9–16	2+	0
6. P. Q.	M	23	40	18	?	2+	0
7. C. K.	M	73	50	23	?	?	35
8. J. D.	M	53	100	48+	24–30	½	386

* Patients received no food during test except glucose per os as required to prevent hypoglycemia.

† The end of activity was considered to occur when the blood-sugar level rose, or remained stationary for several hours.

The duration of action of a given dose of protamine zinc insulin varied from patient to patient as in the depancreatized dog. Illustrative findings are summarized in Table 2. It is apparent from these results that there is a difference in duration of action in the mild diabetic as contrasted with the severe diabetic. In mild diabetics doses of 30 units or more lasted over 24 hours and required large amounts of glucose (by mouth), to prevent hypoglycemia. This is illustrated by Patient A. C. (No. 3, Table 2) where 30 units lasted over 24 hours and required 175 gm. of glucose to prevent hypoglycemia. Severe diabetics, so classified because of a fasting

insulin requirement,[7] showed a much shorter duration of action of comparable size doses. Thus, 40 units in Patient P. Q. (No. 6, Table 2) and J. S. (No. 5, Table 2) with high basal insulin rates, 2+ units per hour, lasted only 18 hours and required no glucose to prevent hypoglycemia.

Table 3 shows a detailed record of the results of injection of 30 units of protamine zinc insulin in a moderately severe diabetic, and Table 4 the effect of 40 units of protamine zinc insulin in a severe diabetic. Tables 3 and 4 illustrate how the results in Table 2 were obtained.

TABLE 3.—DURATION OF ACTION OF 30 UNITS PROTAMINE ZINC INSULIN IN MODERATELY SEVERE DIABETIC (M. F.), Fe 43.*

Time.	Insulin.	Blood sugar in mg. per 100 cc.
7 A.M.	30 PZI H	136
8 A.M.	...	155
9 A.M.	...	155
10 A.M.	...	144
12 NOON	...	125
3 P.M.	...	101
6 P.M.	...	136
9 P.M.	...	112
12 M.	...	86
7 A.M.	...	69†

* Patient fasting throughout test.

† The duration of action is over 24 hours, although the test was stopped at the 24th hour.

TABLE 4.—DURATION OF ACTION OF 40 UNITS OF PROTAMINE ZINC INSULIN IN A SEVERE DIABETIC (J. S., male, 16).*

Time.	Insulin.	Blood sugar in mg. per 100 cc.
6:50 A.M.	...	172.4
7:15 A.M.	40 units	
10:40 A.M.	...	142.8
12:55 P.M.	...	163.9
3:00 P.M.	...	153.8
8:00 P.M.	...	142
11:00 P.M.	...	133
1:16 A.M.	...	140
6:40 A.M.	...	206.2

* Patient fasting throughout test.

The end of action of the 40 units was considered to occur between the 18th and 24th hours.

The results tabulated in Table 2 also show that the peak of activity varied with the size of the dose and the basal insulin requirement, and appeared later with the larger size doses. (The peak of activity was considered to occur when there was the fastest rate of fall of the blood sugar or when the largest amount of glucose was required to prevent hypoglycemia.) To illustrate, 10 units of protamine zinc insulin in Patient F. B. (No. 1, Table 2) with a basal rate of $1\frac{1}{4}$ units per hour had its peak in 6 to 8 hours, 30 units in Patient M. F. (No. 4, Table 2) with a basal insulin requirement of 1+ units per hour was probably reaching its peak at the end of 24 hours, while 100 units in Patient J. D. (No. 8, Table 2) with a

basal requirement of $\frac{1}{2}$ unit per hour, reached its peak between the 24th and 30th hours.

TABLE 5.—DURATION OF ACTION OF PROTAMINE ZINC INSULIN WITH DIET.

Patient.	Sex.	Age.	PZI units (before breakfast).	Duration of* action in hrs.	Diet, C., P., F.
1. G. B.	M	20	45	18–24	250–75–100
2. N. S.	F	14	50	24+	
3. J. D.	M	55	100†	21	250–85–60
4. C. A.	F	..	55	24+	150–65–80
5. C. McF.	F	24	100	24+	150–65–80

* Activity was taken as being at an end if the blood sugar rose from midnight to 7 A.M. (Dinner at 5 P.M.) Blood sugar determinations were made every 4 to 6 hours or oftener during the entire 24-hr. period.

† In two injections of 50 units.

TABLE 6.—PROTAMINE ZINC INSULIN WITH DIET.

(C. A. Colored Female.)

Time.	Blood sugar, mg. per 100 cc.	Diet, C. 150, P. 65, F. 80.	Urine sugar in gm.	Insulin.
6:30 A.M.	..	...	...	55 PZI
7:00–7:15 A.M.	..	Breakfast		
9:05 A.M.	258			
10:45 A.M.	200			
8:30–10:45 A.M.	..	...	300 cc. = no sugar	
10:30–11:00 A.M.	..	Lunch		
1:05 P.M.	370			
3:55 P.M.	266			
10:45–3:55 P.M.	..	...	1390 cc. = 6.8 gm.	
4:30–5:00 P.M.	..	Dinner		
7:40 P.M.	275.8			
3:55–7:45 P.M.	..	...	500 cc. = 2.25 gm.	
11:15 P.M.	248.4			
7:45–11:30 P.M.	..	...	900 cc. = 3.06 gm.	
5:40 A.M.	138.9*			
11:30 P.M.–8:15 A.M.	..	...	1200 cc. = no sugar	
			Total = 12.11 gm.	

* Patient complaining of insulin reaction, given orange juice.

The results of curves of protamine zinc insulin with the diet are summarized in Table 5. Table 6 shows a sample of the type of curve from which the summaries in Table 5 were made. It is apparent from our results that it is difficult to gauge duration of effect or peak of activity with diet, since in 3 of the cases (N. S., No. 2, Table 5; C. A., No. 4, Table 5; C. McF., No. 5, Table 5) the blood-sugar level was falling at the end of the 24 hours. The postprandial rise of the blood sugar after meals (see Table 6) masks the point activity of protamine zinc insulin during the day, so that few deductions can be drawn concerning time of maximum activity. The only period for observation is the postabsorptive period at

night. If the blood sugar falls during this period and then starts to rise abruptly one can make some deductions concerning the duration of action of protamine zinc insulin.

Discussion. When one examines critically many of the curves in the literature the reasons for the divergences of opinion are apparent: 1, Confusion exists concerning the interpretation of curves with diet, as contrasted with fasting curves. 2, No differentiation is made between the duration of measurable activity of a given dose in the severe diabetic as contrasted with the mild diabetic.

The usual curve presented to show duration and peak of activity is done with protamine zinc insulin and diet. From curves of this type few deductions can be drawn concerning peak of action or maximum activity, due to the occurrence of postprandial hyperglycemia. Only when curves are made with the blood sugar maintained in the physiologic range (80 to 150) can deductions be made concerning peak of activity. It is to be expected that the blood sugar will fall when food ceases to be absorbed at night, if there is any appreciable insulin activity present.

The majority of curves adduced to show that protamine zinc insulin has the same effect whether given in the morning or in the evening are in relatively mild diabetics or involve such large doses that activity lasts over 24 hours. As doses of 30 units last over 24 hours in mild diabetics it is obvious that cumulative effects smooth out the curve. That protamine zinc insulin does not have a constant effect in severe diabetics has been shown in Table 2. The difference between activity in the mild diabetic and the severe diabetic needs to be stressed again in this connection. It is important to give protamine zinc insulin to the severe diabetic properly timed in relation to the period when maximal insulin effect is needed. Many of the curves in the literature if examined show the points we are stressing. In the curves of Kerr and Best[4] in the depancreatized dog although the blood-sugar levels were very low an obvious difference in duration of effect with different size doses is apparent. Thus the blood sugar started to rise after 8 hours with 1 unit per kilo of protamine zinc insulin, after 18 hours with 2 units of protamine zinc insulin per kilo and after 28 hours with 3 units of protamine zinc insulin per kilo and there was a severe reaction at 28 hours with 4 units of protamine zinc insulin per kilo.

Summary. We have shown in the depancreatized dog the following facts: 1. The duration of action of protamine zinc insulin varies with the size of the dose, but is not proportional to the size of the dose.

2. The duration of measurable effect varies with the basal insulin requirement of the animal. A dog with a high hourly basal insulin requirement has a shorter duration of action of a given dose than a dog with a low hourly basal requirement.

3. The peak of activity varies slightly with the size of the dose, but the total activity is greater with the larger size doses.

Applying these facts to diabetic patients, the following conclusions were reached: 1. There is a difference in duration of action of a given dose of protamine zinc insulin in the mild diabetic, with no basal insulin requirement, and in the severe diabetic with a basal requirement. The duration of action of protamine zinc insulin in the severe diabetic is influenced by the basal insulin requirement of that individual.

2. There is a period of maximum activity with all size doses of protamine zinc insulin and this period is delayed with the larger size doses.

3. Curves of activity with diet are difficult to evaluate unless rigid criteria are used. A falling blood sugar in the postabsorptive period at night gives little information either as to intensity of activity or duration of activity of a given dose. A falling blood-sugar level followed by a rising level will give information as to the duration of action.

4. The importance of these results in proper timing of insulin dosage in the severe diabetic is discussed.

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OBSERVATIONS ON THE ORAL ADMINISTRATION OF CITRATED BLOOD IN MAN.

II. The Effect on The Stools.*

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In the management of patients with hematemesis or melena it is important to determine the severity of the hemorrhage and whether

* Read by title before the meeting of the American Society for Clinical Investigation, Atlantic City, May 5, 1941. This work was aided by a grant from Parke, Davis & Co., through the coöperation of Dr. E. A. Sharp.

† Justin A. Rollman Fellows in Internal Medicine, 1938–1939, and 1937–1938, respectively.

or not the bleeding has stopped. In this connection one frequently finds the clinician assuming that the patient has had a severe hemorrhage because he has passed a tarry stool, and that the patient is still bleeding because he continues to pass tarry stools or because his stools continue to give a positive chemical test for occult blood. In order to test the validity of these assumptions it was decided to give patients and normal controls known amounts of citrated human blood either orally or by stomach tube and to observe the effect on the stools.

The Amount of Blood Necessary to Produce a Tarry Stool. The subjects used in this study consisted of 3 normals and 15 ward patients varying in age from 23 to 72 who, with one exception, were free of digestive tract disease. Citrated venous blood, 2 to 3 weeks' old, was mixed with 100 to 200 cc. of charged artificial Vichy water* to help disguise the taste and was then administered orally. Before the blood was given to any subject, at least two of his stools were found to be of normal color. A regular diet was allowed, and drugs known to discolor the stools were prohibited. When blood was given more than once, an interval of 10 days was allowed to elapse before the succeeding dose was administered. When a tarry stool was once obtained, no more blood was given.

TABLE 1.—THE AMOUNT OF CITRATED BLOOD REQUIRED TO PRODUCE A TARRY STOOL.

Amount of blood administered (cc.).	No. of subjects receiving blood.	No. with tarry stools.	No. without tarry stools.
50	7	0	7
75	9	0	9
100	7	4	3
125	3	1	2
150	4	1	3
175	2	1	1
200	2	2	0

It will be seen from Table 1 that tarry stools were obtained in individuals given from 100 to 200 cc. of blood. The 4 individuals who passed a tarry stool after 100 cc. of blood did not pass such stools when given 50 or 75 cc. of blood, while the other 5 who passed tarry stools did not do so when given at least one smaller dose than the one which produced a tarry stool. These values are higher than those of 50 to 80 cc. reported by Daniel and Egan[1] in a study comprising a group of 10 healthy medical students. The difference in results may be explained either by the fresh blood used by these authors or by a less rigid criterion of a tarry stool. The designation

* Kindly furnished by the W. T. Wagner's Sons Company.

"tarry" was applied by us only to a glistening stool having the same black color as found on a standard paint color chart.*

Appearance and Duration of Bloody or Tarry Stools After Intragastric Administration of Citrated Blood. The blood used in this phase of the study had been stored in the blood bank at the Cincinnati General Hospital for 3 weeks and was kept at room temperature for an hour before it was administered. The blood was given by stomach tube in quantities of 1000 to 2000 cc. With amounts of 1000 cc. it was allowed to flow by gravity into the fasting stomach through a Rehfuss tube during a period of 30 to 60 minutes. When a total of 2000 cc. was given, the blood was divided into amounts of 700, 700, and 600 cc. and was introduced at 4-hour intervals. A hypodermic injection consisting of codeine sulphate, gr. 1, and atropine sulphate, gr. $\frac{1}{150}$, preceded the administration of the blood in order to prevent too rapid passage through the intestinal tract.

TABLE 2.—APPEARANCE AND DURATION OF BLOODY OR TARRY STOOLS AFTER INTRAGASTRIC ADMINISTRATION OF CITRATED BLOOD.

Subject.	Amount of blood given (cc.).	Appearance of 1st bloody stool (hrs.).	Appearance of 1st tarry stool (hrs.).	Duration of bloody or tarry stools (days).	No. of bloody or tarry stools.
W. S.	1000	12	..	1	5
S. W.	1000	17	..	1	3
F. O.	1000	4	..	3	4
J. G.	1000	9	20	3	5
C. E.	1400	4	24	5	8
F. F.	2000	..	20	4	3

Table 2 shows that following the administration of such quantities, the blood may appear in the stools within 4 hours and that the patient's stools may be entirely bloody and never tarry, presumably as a result of intestinal hypermotility. Three of the 4 subjects receiving 1000 cc. of blood had no tarry stools whatever, the stools possessing a reddish cast following which they became a dark brown. Thus a grossly bloody stool does not necessarily indicate that the blood is entering the intestinal tract low in the small intestine or in the colon.

The number of bloody or tarry stools is not directly related to the amount of blood ingested, since, for example, W. S. had 5 bloody stools after 1000 cc. of blood, whereas F. F. had but 3 tarry stools after 2000 cc. of blood, while C. E. had 8 bloody or tarry stools after 1400 cc. of blood. Bloody stools may be passed for as long as 3 days and tarry stools for as long as 5 days after the administration of a single quantity of blood.

It was of interest that none of the subjects vomited the blood.

* In a previous report,[3] we stated that "we have occasionally obtained tarry stools following the oral administration of 75 cc. of blood, but in order uniformly to obtain tarry stools, we have had to give 100 to 150 cc. of blood." At this time our criterion of a tarry stool was not as rigid as the one referred to in this paper, and the number of observations was less.

Duration of a Positive Test for Occult Blood in the Stools After Oral or Intragastric Administration of Citrated Blood. Hesser[2] made the interesting clinical observation that following hematemesis or melena in patients with bleeding peptic ulcer, occult blood is usually present in the stools for a period of 2 to 3 weeks. (It might disappear within 1 week or persist for as long as 5 to 6 weeks after hemorrhage.) Our observations listed in Table 3 are largely in keeping with this author's observations, since following the administration of a large dose of blood, the stools may show a positive test for occult blood for as long as 14 days. The fact that Hesser obtained positive tests for longer periods may be explained by his larger number of observations (72 patients), by the fact that in some of his patients bleeding may have continued or recurred after admission to the hospital, and by slowing of the intestinal motility in his cases as a result of bed rest and restricted diet.

TABLE 3.—DURATION OF A POSITIVE TEST FOR OCCULT BLOOD IN THE STOOLS AFTER ORAL OR INTRAGASTRIC ADMINISTRATION OF CITRATED BLOOD.

Subject.	Amount of blood given (cc.).	Method of administration.	No. of stools giving positive test with		Duration of positive test (days) with	
			Benzidine.	Guaiac.	Benzidine.	Guaiac.
J. T.	125	Oral	3	3	3	3
	175	Oral	4	3	5	2
	250	Oral	7	7	10	10
G. R.	75	Oral	4	2	4	2
	125	Oral	3	2	5	3
S. W.	1000	Intragastric	7	9	4	5
F. O.	1000	Intragastric	11	10	8	8
C. E.	1400	Intragastric	13	10	11	11
F. F.	2000	Intragastric	12	10	14	12

The subjects used in this phase of the study were on a meat-free, green vegetable-free diet at the time they were given the blood and had shown preliminary negative tests for occult blood in the stools.

Summary. A tarry stool may result from the ingestion of 100 cc. or more of citrated human blood. Following the intragastric administration of 1000 to 2000 cc. of citrated human blood, gross blood may appear in the stools and tarry stools may continue for 5 days and may number as many as 8. Positive chemical tests for occult blood in the stools may persist for as long as 10 days after ingestion of 250 cc. of citrated human blood or for 12 days after intragastric administration of 2000 cc.

Conclusions. The passage of a tarry stool does not necessarily indicate the occurrence of a severe hemorrhage into the digestive tract. The persistence of tarry stools or occult blood in the stools does not necessarily indicate the continuation of such hemorrhage.

We wish to express our thanks to Dr. Paul Hoxworth, Director of the Red Cross Transfusion Service, for his coöperation in this study.

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CALCIUM BILE.

A Clinical and Pathological Study.

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Less than a half dozen clinical reports have appeared in the American and foreign literature in regard to calcium bile. The earliest report of white bile was made by Churchman[1] in 1911, who reported that an analysis of the gall bladder contents revealed calcium salts. Volkmann[11] in 1926 was the first observer to use the term "milk of calcium." In 1931, Phemister[9] and his coworkers made an analysis of 7 patients with calcium carbonate gall stones, 5 of whom were subjected to operation. This was followed in 1933 by Knutsson's[3] group of 12 patients showing calcium carbonate deposits in the gall bladder. Kornblum and Hall[4] in 1935 outlined the roentgenologic findings in a series of 5 patients seen at the Hospital of the University of Pennsylvania.

We have been able to survey the gall bladder material seen in the New York Hospital during the past 8 years which, subsequently, proved to be calcium bile at operation and was confirmed by study of the surgical specimen. During this period we have found 15 patients who fulfilled our criteria, and the specimens from whom were examined in the pathologic laboratory.*

It has been interesting to scrutinize these patients from the standpoint of sex distribution, duration of symptoms, icteric index, biliary study, roentgenologic findings, pathologic summation and follow-up study after 1 year. Table 1 gives a brief summary of these data.

It is readily observed that the age and sex distribution is not unlike that found in the usual gall bladder study. All of our patients complained either of right upper quadrant or epigastric distress with excessive gas. There was not a single instance of jaundice noted in the clinical histories, nor was there any evidence of clinical jaundice as shown by the icteric index studies. Biliary drainage was performed in 10 of the patients, and in none of these trials was a B-fraction obtained. A history of typhoid fever was not elicited in a single instance. Bile cultures taken after operation were negative in 8 patients, and not carried out in the others.

* We are indebted to Dr. N. Chandler Foot for the use of his histologic material.

Only 1 of our patients was acutely ill at the time of hospitalization. At operation the cystic duct was found occluded, constricted, or surrounded by indurated tissue in every patient. The mucosa was absent in 4 specimens (Fig. 1) and evidence of chronic cholecystitis was seen in all microscopic sections (Figs. 1 and 2). In this series of patients, we could not demonstrate hypertrophy of the muscularis as described by Knutsson.[3] The calcium carbonate paste was occasionally stained with bile pigment. In 1 instance, the paste was described as black and in 5 others the material was chalky white. Chemical examination carried out in 1 instance showed 76.9% calcium carbonate.*

TABLE 1.—RÉSUMÉ OF THE CLINICAL AND PATHOLOGIC STUDY.

Case No.	Age.	Sex.	Duration (yrs.).	Icteric index.	B. bile.	Lesions found.	Follow-up (1 yr.).
1	54	F	3	8	None	Stone in cystic duct Chr. cholecystitis	Well
2	48	F	4	6	None	Cystic duct small Chr. cholecystitis	Well
3	50	M	4	10	Unknown	Stone in cystic duct Mucosa absent	Well
4	51	M	3	12	Unknown	Cystic duct constricted Mucosa absent	Well
5	33	M	2	5	None	Cystic duct small Chr. cholecystitis	Well
6	50	F	8	6.8	None	Stone in cystic duct Mucosa absent	Well
7	58	F	3	7	None	Cystic duct small Mucosa absent	Well
8	20	F	1	Unknown	None	Calcium injected in wall	Well
9	41	F	2	6	Unknown	Stone in cystic duct Chr. cholecystitis	Well
10	45	F	3	7.5	None	Cystic duct small Strawberry gall bladder	Duodenal ulcer
11	49	F	3	Unknown	None	Cystic duct surrounded by indurated tissue Chr. cholecystitis	Well
12	46	F	1	6	Unknown	Stone in cystic duct Chr. cholecystitis	Well
13	35	F	1	5	Unknown	Stone in cystic duct Subacute cholecystitis	Well
14	31	F	5	5	None	Stone in cystic duct Chr. cholecystitis	Well
15	44	F	2	6	None	Stone in cystic duct Chr. cholecystitis	Well

It goes without saying that a single chemical examination of 1 specimen fails to prove that all the specimens contained calcium carbonate. As we proposed this investigation to be a clinical one, we did not deem it essential to carry out further chemical analyses. Furthermore, every patient was independently diagnosed by the roentgenologist and, subsequently, by the pathologist.

* The specimen weighed 2.2915 gm. and 3 days later 2.2755 showing a loss of 16 mg. on standing due to evaporation (drying). The specimen was mixed about 250 cc. of water and allowed to stand 67 hours, in the hope that all the calcium carbonate would settle and that the nearly clear fluid might be decanted and filtered. The deposit weighed on drying, 1.7 gm. An aliquot part of the liquid was treated with HCl, evaporated and then incinerated. The result indicated that 250 cc. yielded (or would have yielded) 200 mg. of calcium chloride, corresponding to 190 mg. of calcium carbonate ($CaCO_3$). A part of the dried residue weighing 500 mg. was converted into the chloride and incinerated. The ash weighed 505 mg., corresponding to 459 mg. of calcium carbonate; hence the 1.7 gm. contained 1.56 gm. of calcium carbonate. This added to the 190 mg. obtained from the decanted liquid equals 1.75 gm. or 76.9% of the original weight of the specimen—2.2755 gm.

A preoperative diagnosis of milk of calcium bile is strictly radiographic. If and when a scout film (Fig. 4) is made, the diagnosis is relatively simple. Here we find a calcification in the upper right quadrant which is to be differentiated from other calcifications occasionally found on the flat film such as calcified nodes, cysts, stones,

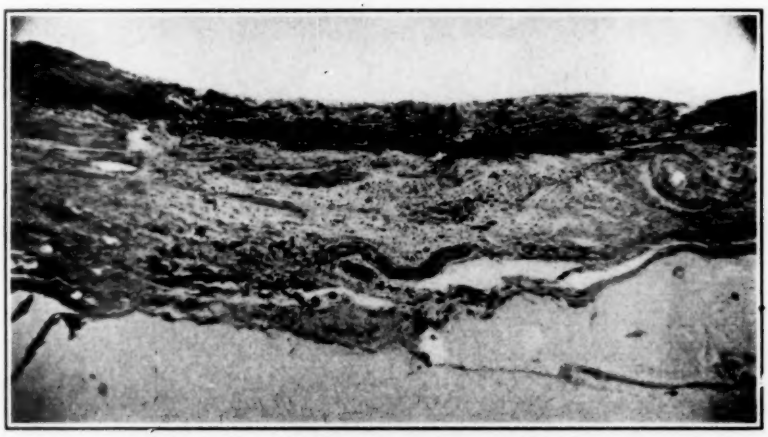

FIG. 1.—(No. 138959.) Gall bladder with no cellular exudate in submucosa. Only a few strands of muscle tissue remain.

FIG. 2.—(No. 244379.) Gall bladder shows an increase in fibrous tissue throughout with many lymphocytes and leukocytes. There was no muscular hypertrophy.

FIG. 3.—Pathologic specimen. Before operation the films showed a smooth shadow which did not change in size following the high fat meal.

FIG. 4.—(No. 138959.) Shadow is due entirely to limy bile taken before the dye was ingested.

calcified gall bladder, or unilateral costal cartilage calcification. The limy bile shadow is quite homogeneous except when negative stones are present. The density varies, depending upon the calcium content, and the shadow may be barely perceptible or quite dense. If the calcium material is thin, the opaque shadow may change in shape from day to day but it will always be present. In case a preliminary scout film is not made, there is often difficulty in deciding whether the shadow seen is that of the dye-filled gall bladder

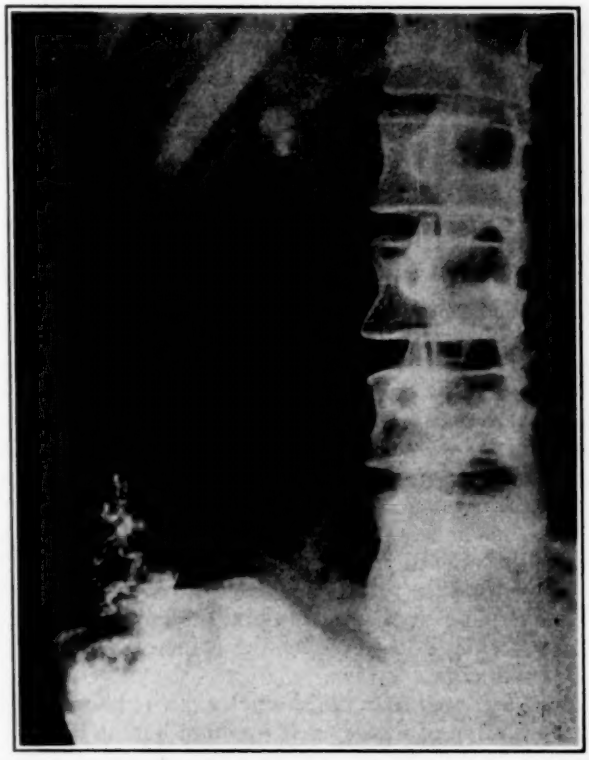

FIG. 5.—Shadows of calcium density in the region of the cystic duct and in the fundus.

or of calcium carbonate bile. The first suggestion that calcium bile may be present is noted when the gall bladder does not decrease in size following ingestion of a high fat meal. This in itself is not pathognomonic. Many gall bladders are seen which have not changed their size on the postfatty meal film, but in this case a subsequent flat film of the gall bladder should be taken. This is particularly true when either definite or questionable stone shadows are seen in the region of the cystic duct (Fig. 5).

The presence of calcium bile should be suspected when a visible gall bladder of calcium density is seen without cholecystography. This impression may be further fortified if the gall bladder is visible and an opaque stone is noted in the cystic duct. A gall bladder containing milk of calcium bile often shows no change before or after the fat meal. Atypical shadows unlike gall stones which are found in association with a non-visualized gall bladder during cholecystography should be regarded with suspicion. The diagnosis of calcium bile is entertained when: 1, a visualized gall bladder is seen in roentgenograms without cholecystography or observed roentgenoscopically during a gastro-intestinal study; 2, no change in the appearance of the gall bladder is seen in the cholecystogram after a fatty meal; 3, there is a change in the shape of the visualized organ; 4, gall bladder shadow persists after cholecystography; 5, a stone in the cystic duct is seen with a visualized gall bladder; 6, when shadows unlike gall stones are found in association with a non-visualized gall bladder during cholecystography.

Discussion. The formation of calcium bile and carbonate gall stones are probably closely related. Wilkie[12] was able to reproduce calcium calculi in rabbits. He found that upon ligation of the cystic duct in the presence of cholecystitis that calcium stones were formed. He concluded that the cystic duct must be occluded by some process before certain changes occur in the physiology of the gall bladder leading to subsequent mucosal effects in the duct and the wall of the organ. In due time, he concluded, there would be an increase in the excretion of calcium from the gall bladder. We believe that a chronic inflammatory process with partial obstruction of the cystic duct is essential to calcium carbonate formation. Whether the calcium is derived from the gall bladder wall is an unsettled question, although Phemister[9] and his coworkers were able to demonstrate the presence of calcium only in the gall bladder side of stones found in the cystic duct. Schubb and Goodstone[10] believe that calcium in the gall bladder is increased in the presence of inflammation alone.

Lichtwitz[5] suggested that the character of the bile reaction influenced the formation of the deposits found in human bile. Naunyn[6] advanced the view that a *special* biliary reaction may be necessary for carbonate deposition. Okada[8] noted the difference in reaction between gall bladder bile and liver bile in the dog, as did Neilson and Meyer[7] for the rabbit, and these observations were largely confirmed by Drury, McMaster, and Rous.[2] The latter state that normal liver bile is alkaline with an average pH of 8.2; but in the gall bladder, the secretion undergoes a change toward the acid side with a pH of 5.18 to 6. It would appear, therefore, that when a gall bladder has been damaged by any cause, it may be unable to alter the character or the pH of the bile coming from the liver. This change may play a rôle in the precipitation of calcium salts.

Therefore, no definite conclusion can be drawn as regards the etiology of calcium bile. Its secretion from the wall of the gall bladder, as assumed by Phemister,[9] has not been proved by any experiment so far as we know. When the cystic duct is partially occluded by stone, or by inflammatory induration, it is possible that the organ may develop abnormal physiologic functions. It is more likely, however, that the precipitation of calcium bile occurs subsequent to the reduction in the cholesterin and bile-salt content of the bile which, in turn, is secondary to the inflammatory changes seen in all of our specimens. It was Knutsson's[3] belief that the wall of the gall bladder was hypertrophied, due to obstruction in the duct, but we were unable to demonstrate such a change in our specimens. On the other hand, we do agree with his assumption that the calcium salt is precipitated from the bile itself when the outflow from the gall bladder is hampered by obstruction of the cystic duct. This phenomenon occurs only when certain mucous membrane changes have interfered with the absorptive and excretory functions of the organ.

Summary. We have reported 15 patients with calcium bile demonstrated by scout films and corroborated by examination of the surgical specimens. The clinical facts have been outlined and the salient features noted in the histologic study have been discussed. Finally, various theories to explain this disturbance of calcium metabolism in the bile have been presented.

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THE DIAGNOSIS, INCIDENCE, AND SIGNIFICANCE OF ESSENTIAL ACHLORHYDRIA.

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In recent years, there has arisen a renewed interest in the subject of achlorhydria. This is chiefly due to the demonstration by Castle and his coworkers of an "X" substance in gastric secretion

closely related to pernicious anemia. However, achlorhydria is not only of importance in pernicious anemia, but is also prominently involved in the following conditions: gastric carcinoma, chronic gastritis, gall bladder disease, Graves' disease, tuberculosis, gastrogenous diarrhea and other gastrogenous intestinal disturbances; chronic arthritis, alcoholism, subacute combined sclerosis, anorexia nervosa, sprue, pellagra, gastric lues, Simmonds' disease and achlorhydric anemias. A complete discussion of the significance of these achlorhydrias would take us too far afield. Bloomfield and Polland[2] have discussed the entire subject in a monograph to which the reader is referred.

The terms "anacidity," "achlorhydria" and "achylia gastrica" have been used loosely in the past. "Anacidity" should be dropped since practically every sample of gastric contents is on the acid side of pH 7. "Achylia gastrica," a term introduced by Max Einhorn, signifies an absence of gastric secretion. Since gastric secretion may contain free hydrochloric acid, pepsin, rennin, lipase, mucin, some diluting secretion, and the intrinsic "X" substance of Castle, it is doubtful if true achylia gastrica ever occurs. The term might properly be retained for absence of free acid and ferments, provided such a definition is given specifically by each author.

The word "achlorhydria" means the absence of free hydrochloric acid. The subject of achlorhydria is, even at the present time, in a very confused state. Precisely what is meant by achlorhydria is neither clear in the theoretical discussions nor in practical clinical medicine. Certain questions arise. How is the achlorhydria diagnosed: by what type of test meal, by what indicators? Do we mean by achlorhydria a failure to react to Toepfer's solution, or to color Congo red paper blue? Do we mean a certain pH (3.5) or over? Are we interested in the achlorhydria that may occur in an individual's stomach during the normal course of digestive events in his life (that is, during, after, and between his usual meals)? Are we concerned with transient, temporary achlorhydria, or, are we considering only permanent achlorhydria? In other words, is it the false or transient ones and even the relative subacidities that interest us, or, is it the absolute, true, complete, and permanent achlorhydrias? From these questions alone, the complexity of the problem is patent. Probably it will be necessary to restudy the old investigations and institute new ones in order to answer these questions. Perhaps all of them are of importance and require emphasis and analysis.

From the heterogeneous group of these achlorhydrias we have selected one type for study, namely, the true or absolute achlorhydrias. By a "true achlorhydria" we mean a pH of 3.5 or over, one that is complete, and more or less permanent; in other words, a state in which the stomach is incapable of secreting free hydrochloric acid even under maximal stimulation.

At this point we will review briefly the work of other recent

investigators. We agree with these authors in their main contention that true achlorhydria should and does occupy an important place in present-day clinical medicine. We differ from them in the normal incidence of this condition when it is unassociated with ascertainable organic disease. Their high figures, we believe, are largely due to the inadequate methods used in investigating patients with apparent achlorhydria.

Certain writers (Zagal, Marks and Kantor[9]) using the Ewald test meal, without a fractional meal or a histamine test as a control, give the incidence of achlorhydria as follows: In 7679 cases studied, 564 (7.3%) had achlorhydria. The incidence increased from 2% to 5% in the first three decades of life to 16% to 22% in the sixth, seventh, and eighth decades. Using a modified Ewald test meal continued for 2 hours, a careful study of the achlorhydria cases was reported from the Mayo Clinic. In a study of 3746 records, Vanzant and collaborators[6] reported the incidence of 12% and the figures ranged from a 4% occurrence at the age of 20, up to 23% to 26% in the aged. This very high incidence may be due to a difference in the material or more probably to the method of study—usually the Ewald test meal. As was stated in their article, "unfortunately the control study with histamine was made in only a small per cent of the cases." In the study of achlorhydrias reported by Bockus, Bank and Willard[3] their incidence was 5.7% in a series of consecutive gastro-intestinal cases. However, if one removes from this group the cases which secreted free acid after histamine and also the cases with a recognized etiology, the incidence of true achlorhydria of unknown etiology drops in their series to 2.2%.

We are presenting our experience with this particular phase of the subject for the following reasons: 1, our cases have been studied repeatedly; 2, the method of study differs from those previously used; 3, our results differ strikingly from those usually given in the literature; 4, using these methods of study, it is possible to isolate and describe with greater accuracy the separate group of "true achlorhydrias."

We will next discuss the methods of studying the stomach for the presence or absence of the acid secretion. The diagnosis of achlorhydria has successfully passed through the stages of the Ewald test meal, of repeated Ewald test meals, the Riegel test, the various types of the fractional test meal, the histamine test, the combined tests (urinary and gastric acidity studies) and the chromoscopic procedure (neutral red). The ideal method of establishing the fact as to whether the stomach is capable of secreting free hydrochloric acid under the usual daily conditions of living would be to leave an indwelling catheter in the stomach 24 hours daily and, using either constant suction or repeated aspirations, or, preferably, an indwelling H-ion electrode, test the gastric contents for free acid throughout the 24 hours of the day. We have already studied the

gastric secretion throughout the 24 hours in some patients with duodenal ulcer while on the Sippy treatment to ascertain the degree of neutralization throughout the day and night. Also, in a large series of cases, we approximated this ideal by doing a 3-hour oatmeal gruel fractional test meal during the day, plus a fractional study of the gastric secretion throughout the night. These studies (reported elsewhere[7]) are, of course, impractical for the investigation of ambulatory private and outpatient department patients. Also, they do not answer the question in which we are chiefly interested, as to whether the stomach is incapable of secreting free hydrochloric acid at any time under all conditions and especially under the influence of the greatest stimulation to secretion. After working with several methods, chiefly the oatmeal gruel and alcohol fractional test meals, the histamine test and the neutral red test, we arrived at a combined method which we believe is a satisfactory test for the ability of the stomach to secrete free hydrochloric acid.

In evolving this test we have considered the present state of our knowledge of the physiologic mechanisms involved in gastric secretion (Babkin[1]). The normal human stomach is first stimulated to secrete over nervous pathways. The thought, sight, taste, smell and odor of food acts as a stimulus to the fundus glands through the vagus nerve and accounts for a considerable amount of the gastric secretion. Following this initial stage (or even without it) certain secretagogues and food substances (split and unsplit) act in and through the antrum to produce some substance (probably a hormone) which after absorption act without nervous intervention through the blood stream on the fundus glands and account for the prolonged gastric secretion which is necessary to complete gastric digestion. While the upper small intestine is chiefly the site of inhibitory stimuli, a certain amount of gastric secretion is produced (also chemically) through intestinal stimuli. As a result of these considerations it is necessary to have a test which will stimulate the psychic and chemical phases maximally. For various reasons oatmeal gruel was chosen as the test meal. It is considered an efficient stimulus to the nervous phase of gastric secretion and in itself neither neutralizes nor absorbs free acid nor does it stimulate mucin excessively. Also, it has been used so widely by numerous workers that it affords a better basis of comparison than other test meals.

Histamine was employed simultaneously. This is a powerful direct chemical stimulant of the acid-secreting parietal cells. Since we had confirmed the original work of Glaessner with neutral red, we included this test. In a previous study[8] we had found that when neutral red is injected intramuscularly it will not appear in the gastric contents within 2 hours if the stomach cannot secrete free acid. This dye is a specific indicator of the ability of the parietal cell to secrete acid. Thus, by combining the three tests,

food, histamine, and neutral red, we subject the acid cells to a good food stimulus, to a powerful chemical incitant, and to what seems to be an excellent indicator of cell function. While still not entirely ideal from the theoretical point of view, this type of combined test for gastric secretory function seems to be the most complete and intensive method of study used to date. The details of the combined test will now be described.

Method. The patient presents himself in the morning in a fasting state. The thin Rehfuss or Levin tube is passed and the fasting contents aspirated. Histamine (Imido-Roche) (0.5 mg.) is injected subcutaneously and, at the same time, 40 mg. of neutral red dissolved in 1 to 2 cc. of sterile distilled water is injected intramuscularly. The patient then drinks 150 cc. of strained oatmeal gruel. A sample is aspirated every 15 minutes for 2 hours in the outpatient department and for 3 hours in the wards of the hospital. The samples are tested for free hydrochloric acid with Congo red paper and for free and total acidity with Toepfer's solution and phenolphthalein as indicators. If free acid is absent with Toepfer's solution, the pH is 3.5 or higher. Each sample is inspected for the presence of neutral red. This appears as a watery pink to a red color. Rarely in the late aspirations, bile, stained reddish-brown by the dye, regurgitates into the stomach. This can be readily recognized by the experienced eye and possibly vitiates the test somewhat, so that it is better in such instances to repeat the procedure. If no free acid is present, each sample may be acidified to bring out the dye. If the dye appears and free acid does not (and this is a very important point) it signifies in our experience, that the stomach is capable of secreting free acid, that the achlorhydria is a temporary or false one (inhibition, insufficient stimulation, or, neutralization of the free acid) and that further tests are indicated. Under such conditions it has been our custom to repeat the test meal with certain modifications. The histamine, provided the reaction to 0.5 mg. was slight, may be increased to 1 mg. The patient is given one-half of an orange to chew and directed to expectorate all the pulp and juice, *i. e.*, a powerful psychic stimulus, and finally warm bouillon or 5% alcohol (75 cc.) is added to the oatmeal gruel meal. The last provide a very strong chemical stimulus. If such a meal fails to reveal free hydrochloric acid, the large Ewald tube may be passed unexpectedly 1 or 2 hours after the patient has eaten his usual meal (breakfast or dinner) at home or on the ward. In this way we have found (see below) that when neutral red appears within the period of the 2-hour test meal, that the stomach is capable of secreting free acid and that we are not dealing with a true achlorhydria. We have concluded as a result of these studies that histamine alone is not sufficient to establish the diagnosis of true achlorhydria.

Material. A routine Rehfuss fractional test meal (150 cc. strained oatmeal gruel with ¼ hour aspirations for 2 hours) was carried out in 5585 consecutive patients in the Gastro-Intestinal Clinic at the Mount Sinai Hospital. Whenever a 2-hour achlorhydria was encountered the combined test described above was instituted.

Results. In 5585 consecutive cases we encountered 117 cases of true achlorhydria (2.2%). If the cases of pernicious anemia, gastric carcinoma, partial gastrectomy, gastric syphilis, chronic gall bladder disease, and Graves' disease, comprising in all 46 examples of diseases usually associated with achlorhydria, are excluded, 69 instances of true achlorhydria of undetermined etiology are left.

This is an incidence of 1.2% in 5585 consecutive test meals obtained from a group of miscellaneous patients attending the Gastro-Intestinal Clinic. In these 69 true achlorhydrias a trace of pepsin was found in all but 2 cases. Complete blood studies are not at hand in all of these cases. Of 11 who were studied hematologically, 8 had a hemoglobin between 85% and 100% and a red cell count of $4\frac{1}{2}$ to 5 millions. Two patients had a mild secondary anemia and 1 had a severe microcytic secondary anemia (45% hemoglobin and $2\frac{3}{4}$ million red blood cells).

The distribution in the sexes and in the different age levels of our cases of true achlorhydria unassociated with definite organic diseases is given in Table I. It is interesting to note that the incidence in males is greater. In view of the fact that an analysis of the total

TABLE 1.—ANALYSIS OF THE CASES OF TRUE ACHLORHYDRIA OF UNDETERMINED ETIOLOGY.

Age.	Male.	Female.	Cases.
0–20	1	1	2
20–30	2	5	7
30–40	5	6	11
40–50	15	6	21
50–60	9	5	14
60–70	10	4	14
Total	42	27	69

number of male and female cases studied was not made, the statistical significance of this sex difference cannot be established. The fourth, fifth and sixth decades show nearly double the number of cases in the first three decades. The extremely small incidence of true achlorhydria of unknown etiology in our series is indeed striking. It is our impression from prolonged observation of some of these cases, that despite intensive therapy, the free acid rarely returns.

We were dissatisfied with the methods and studies reported when we found in our studies that when neutral red is excreted into the stomach after intramuscular injection, irrespective of whether free acid appears in that test or not, the stomach is capable of secreting free acid and with some modification of the test meal will do so sooner or later. That our ideas in this respect were correct was proved by our experience in 15 cases where histamine ($\frac{1}{2}$ to 1 mg.) plus neutral red plus oatmeal gruel was used as a combined test and neutral red appeared without the presence of free hydrochloric acid. In each instance, at a subsequent test of one type or another, free hydrochloric acid was demonstrated. This is a striking point and possibly invalidates conclusions which have been drawn from "histamine achylias." Apparently, neutral red is a more delicate indicator of the functional activity of the gastric secretory cells than histamine.

Comment. It is apparent, if one excludes the diseases associated with true achlorhydria, that this affection of the stomach is indeed

a rare one. The obvious deduction is that when a physician encounters a patient with a true achlorhydria, it is often associated with a definite disease and it is, therefore, imperative that he search intensively for one of the usual underlying causes or conditions. Whether the patients who do not reveal an associated disease will develop one of these diseases, particularly one of the anemias or carcinoma, as Hurst suggests, depends on a survey of the entire situation. We watch particularly for the onset of pernicious anemia, gastric carcinoma, and gastritis. A history of pernicious anemia or unexplained secondary anemia in other members of the family, familial achlorhydrias, burning sensation in the tongue or objective glossitis, dysphagia, paresthesiæ, lemon-yellow color, subicterus, diarrheas, pallor, weakness, or evidence of spinal cord involvement, should suggest the possibility of pernicious anemia, present or impending. Loss of strength, anorexia, aversion for certain foods, loss of weight, nausea, constipation and anemia should evoke at once the thought of carcinoma. A mild, chronic gastritis without anemia or carcinoma gives, as a rule, no or only mild symptoms. However, epigastric pain, fullness and pressure immediately on and after eating; morning nausea, heartburn, belching; constipation or diarrhea or an alternation of these symptoms; vague diffuse abdominal pain; flatulence and cramps may follow or be reflected from a diffuse inflammation of the gastric mucosa. It seems therefore advisable to watch the stomach of these patients for the changes which give evidence of inflammation, namely, an increase in mucus, hypertrophy of the rugæ in the radiographic examination and certain gastroscopic changes (chiefly atrophy and hypertrophy). It is probable that the near future will reveal an improvement in the diagnosis of chronic gastritis because of increasing skill in gastroscopic methods. Schindler, Moutier, Jackson and others have reported characteristic gastroscopic pictures in chronic gastritis.

It seemed of interest and importance to gastroscope the stomachs of some patients with true achlorhydria of undetermined etiology. Fourteen patients were selected for study.* Summarizing the table, 9 of the patients (64%) showed a diffuse atrophic gastritis; 4 showed a hypertrophic gastritis; in 2 of these the lesions were diffuse and in 2 were located in the antrum; in 1 there was a mixed hypertrophic and atrophic gastritis diffusely located. It is apparent that a severe gastritis was present in all of these true achlorhydrias; none was normal. It is of interest that in two-thirds of the cases there was a diffuse atrophic gastritis. *A priori*, one would expect this type of gastritis to be associated with true achlorhydria. Whether it is associated with the development of carcinoma as suggested by Schindler and Hurst will only be answered by careful future studies.

* I am indebted to Drs. Rudolph Kramer and Henry Lerner for the data obtained in these examinations (see Table 2).

This brief study also suggests but, of course, does not prove, that the advanced gastritis noted in most of the patients probably preceded and caused the true achlorhydria.

TABLE 2.—GASTROSCOPIC FINDINGS IN TRUE ACHLORHYDRIA.

	Type of gastritis.			Location of gastritis.		
Case.	Atrophic.	Hypertrophic.	Mixed.	Antrum.	Fundus.	Diffuse.
1	..	+	..	+		
2	+	..	..	..	..	+
3	+	..	..	..	..	+
4	+	+				
5	..	+	..	+	+	
6	..	+				
7	..	..	+	..	..	+
8	..	..	..	..	..	+
9	+	..	..	..	..	+
10	+	..	..	..	..	+
11	+	..	..	..	..	+
12	+	..	..	..	..	+
13	+	..	..	..	..	+
14	+	..	..	..	..	+

Hurst has suggested a prophylaxis of the chronic gastritis associated with the achlorhydric state. In general, from his observations, it would seem advisable in public health work, to advise people from 35 years upward, with or without symptoms, to have a test meal and Roentgen examination and possibly a gastroscopy every 6 to 12 months. Should an achlorhydria or other evidence of gastritis be at hand, one should institute prophylactic and therapeutic measures. For example, diet, gastric lavage (autolavage or by the physician), liver and iron. After some improvement the administration of hydrochloric acid and pepsin may be indicated. While not a cure-all or a perfect prophylaxis, some such plan, Hurst thinks, would possibly, 1, give us earlier diagnoses of pernicious anemia, achlorhydric anemia, gastric carcinoma, chronic gastritis, chronic gall bladder disease, Graves' disease; 2, help to prevent in some measure such anemias as mentioned; 3, cure or retard chronic gastritis and thus, 4, perhaps by decreasing both the atrophic and the hyperplastic reparative phase of the inflammation, lessen somewhat the incidence of gastric carcinoma.

Finally, a few words should be added concerning the etiology of the true achlorhydrias. Hurst[5] feels that all human stomachs are subject to chronic irritants (dietetic, infectious, traumatic, alcohol, tobacco, and so on) and that 80% endure these insults very well. Ten per cent, he believes, will get a "hyperchlorhydric gastritis" which leads to the ulcer disease. Another 10% will develop the subacid or achlorhydric gastritis which may lead to the anemias or to the malignant state. Why these endogenous and exogenous irritants should cause in some patients secretory hyperfunction and in others hypofunction is not clear. Apparently we must fall back with Hurst on the "constitution" idea. While interesting and stimulating, these suggestions require careful study. One demands further

evidence in order to accept this attractive hypothesis. Recently we observed a small group of patients with gastric ulcer with free acid who later developed achlorhydria. This would seem to indicate a progressive gastritis. In other words, the gastritis accompanying ulcer may also lead to the hypochlorhydric state.

Faber[4] in a recent monograph on gastritis reiterates his belief that achlorhydria is due to gastritis. He disagrees with Hurst in that he explains the hypochlorhydric and hyperchlorhydric states on the basis of a difference in the type, degree, and localization of the gastritis rather than on a constitutional basis.

We are inclined to agree with these authors that gastritis is the chief cause of achlorhydria and that, henceforth, the inflammatory states of the stomach should engage our attention closely. There may be many varied causes for the gastritis. In addition to those suggested by Hurst, it seems probable that certain functional states (pylorospasm, retention, atony, hypertonic states, myospasm, vasospasm, acid-pepsin irritation), and toxic-infectious factors (chiefly from the nose, throat and teeth) may play a very important rôle in the genesis of gastritis. We are at present conducting an investigation of the occurrence and diagnosis of gastritis under these various conditions.

Summary and Conclusions. 1. The terms "anacidity" and "achylia gastrica" should be dropped and "false" or "true" achlorhydria be adopted. By "true" is meant one that is complete, more or less permanent and with a pH of 3.5 or over.

2. The Ewald and Riegel test meals, the oatmeal gruel fractional test meal, or the histamine test alone, are insufficient to establish the diagnosis of a true achlorhydria.

3. A combination test including oatmeal gruel, histamine, and neutral red seems more efficient in the diagnosis of true achlorhydria.

4. Using this procedure, we have found that the incidence of true achlorhydria unassociated with the organic diseases usually accompanying true achlorhydria is very low (1.2%), *i. e.*, true achlorhydria of undetermined etiology is rare in individuals, young or old.

5. Inasmuch as true achlorhydria is often associated with definite organic diseases, its presence should lead the examiner to a thorough search for these diseases.

6. In the absence of such diseases certain prophylactic measures against the gastritis probably causing the achlorhydria should be instituted in an attempt to prevent the subsequent development of these diseases.

7. It is possible that the incidence of pernicious anemia, some of the secondary microcytic anemias, various intestinal disturbances, chronic gall bladder and liver disease, various allergic phenomena, and, above all, gastric carcinoma, may be lessened by the proper study, recognition, and prevention of the gastric inflammatory states which lead to subacidity and the achlorhydrias.

8. Finally, the possible etiologic factors involved in the gastritis associated with achlorhydria are briefly reviewed and discussed.

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IMMEDIATE FEEDING VERSUS INITIAL STARVATION IN THE TREATMENT OF BLEEDING PEPTIC ULCER.

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The purpose of this paper is to compare the results of initial starvation followed by a progressive Sippy diet with the Meulengracht regimen in the treatment of 81 patients with bleeding peptic ulcers admitted to this hospital from January 1, 1936, to April 15, 1941, inclusive.

In the majority of these cases, the diagnosis was substantiated by Roentgen-ray, subsequent operation, or autopsy. In only 17 of the 81 cases was the diagnosis a clinical one. No cases were included in which the red blood cell count was not below 4 million. Those cases with concomitant perforation of a peptic ulcer were excluded. Also not included are 6 patients from the Sippy series and 3 patients from the Meulengracht group who were treated by early surgical intervention when they appeared to fail to respond to medical management. One patient died from generalized peritonitis and the others recovered.

Of the 43 patients treated by initial starvation, 37 were males and 26 of the 38 patients who were fed immediately were males. Although males predominate in the former group, there is no reference in the literature to any difference in prognosis between the two sexes. The average age of the patients treated by the Sippy regimen was 44 years and that of the group treated by Meulengracht's method was 46 years. Since the mortality rate rises with increasing age in this disease, the advantage here, though slight, lies with the group treated by the Sippy routine. There were 13 gastric ulcers, 21 duodenal ulcers, and 9 of undetermined location in the patients treated by initial starvation. In the group treated by immediate

feeding the lesions were distributed as follows: gastric, 12; duodenal, 15; marginal, 4; and undetermined location, 8.

Methods. In this hospital, until July of 1939, patients with bleeding peptic ulcers were starved for an initial period averaging 3 days (range 1 to 6 days). They were then started on progressive Sippy diets, often having their food intake further restricted by being kept on the first day Sippy diet for several days. They were given aluminum hydroxide in widely varying dosage. Most of the patients received iron toward the end of their hospital stay. Patients often were given sedatives liberally.

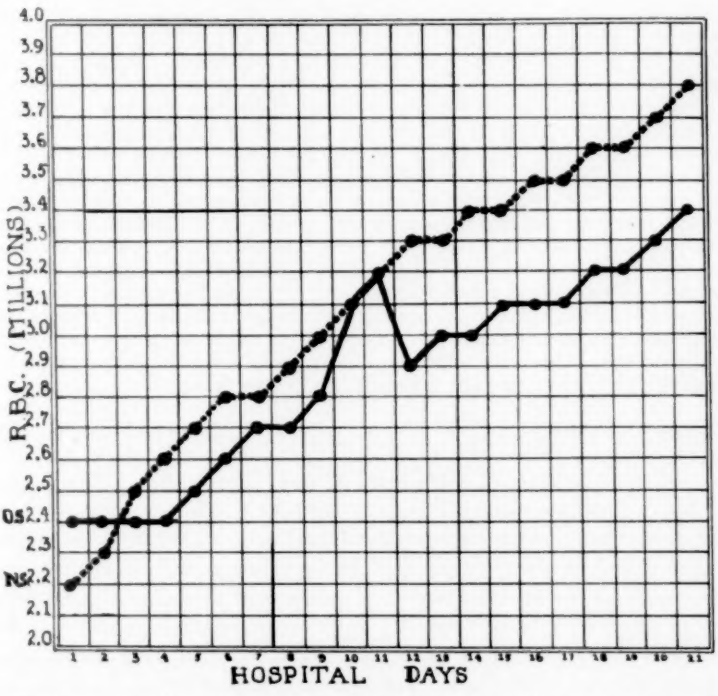

CHART 1.—Average daily erythrocyte count of the patients treated by initial starvation (solid line, O.S.) compared with those treated by Meulengracht's method (broken line, N.S.).

In 1932, Meulengracht[2a,b] of Copenhagen first advocated the system of treatment which bears his name. Meulengracht, who has never referred a bleeding patient for surgery, believed it to be fundamentally wrong to withhold food and drink from patients in a condition of posthemorrhagic shock. He stated as his reasons for advocating the feeding of these patients, first, that patients often die in spite of rigid diet or the complete withdrawal of food; second, that patients have been observed to stop bleeding and recover from their anemia although they remain ambulatory and do not seek treatment; and lastly, that a diet deficient in vitamins and calories does not promote either the healing of ulcers or the regeneration of blood. In analyzing the results of cases treated by the old method he found that most of them died about the eighth day, which was exactly the average number

of hospital days of the fatal cases in this series. He postulated that many of these patients died from sheer exhaustion. He also pointed out that patients were more comfortable and that the nursing care was easier and the hospital stay shortened when the patients were fed. Other authorities have advanced further reasons for the use of liberal feeding. Lanphere,[1] in particular, emphasized that a high carbohydrate and protein intake tends to protect the liver and to prevent acidosis. Others have suggested that free acid in an empty stomach delays the healing of ulcers.

Of Meulengracht's own series of 491 consecutive patients treated by feeding, only 10 died—1 from perforation of his ulcer, 4 before they could be fed, and 5 from recurrent hemorrhage, giving a corrected mortality of 1%.

Since July, 1939, all patients with bleeding ulcers have been treated with frequent nutritious feedings from the time of admission. Patients who vomit food or blood are refed. The foods are selected by the dietetic staff from a large assortment of vegetables and fruits, all cooked and strained, and each patient is given 100 cc. of orange juice daily for the vitamin C content. Cereals, crackers, wafers, and toasted bread are all allowed and eggs are used freely, prepared in any way except by frying. Meat or fish, which is baked, boiled, or broiled, is given daily from the beginning. The patients are given cream soups, any beverage except coffee, and such desserts as custards, puddings, jello, or ice cream. In addition to their diet these patients received 0.7 gm. of ferrous sulphate 3 times a day and 9 4-cc. doses of aluminum hydroxide in 24 hours. Patients are required to take 3000 cc. of fluid daily. Blood transfusions are given freely when the erythrocytes are under 2 million, when there is evidence of shock, or when the blood urea nitrogen is elevated. Of this group of patients, 23 received an average of 865 cc. of blood. Among the surviving patients treated by initial starvation, 21 were given an average of 790 cc. of blood.

Results. Among the 43 patients treated by initial starvation, 8 died, a mortality rate of 19%. In the second series of 38 patients, treated by immediate feeding, there were no deaths. The graph represents the average daily gain in red blood cells of the two groups of cases, excluding the fatal cases. It will be noted that the patients who were fed at once regenerated blood more rapidly than the survivors among the starved ones. The average period of hospitalization of the earlier series was 29 days, as compared to 25 days for the later series. Three patients had hematemeses during the starvation period and more than 24 hours after admission. None of the patients who were fed on admission vomited blood more than 24 hours after treatment was started.

Conclusion. In a series of 81 patients with bleeding peptic ulcers, 43 were deprived of food and 38 were fed immediately. Comparison of the results in the two series indicates that immediate liberal feeding results in, first, a greatly lowered mortality rate, second, more comfort for the patient, and, finally, a shorter hospital stay.

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BOOK REVIEWS AND NOTICES

AN X-RAY ATLAS OF SILICOSIS. By ARTHUR J. AMOR, M.D. (Lond.), M.Sc. (Wales), Honorary Physician, Clydach Memorial Hospital; Medical Officer, Mond Nickel Company, Ltd., Swansee. With Translation of the Legends into French by ROBERT E. HORNE, M.A. (Wales), Medical Secretary, Mond Nickel Company, Ltd., Swansee. With a Foreword by SIR WILSON JAMESON, M.A., M.D. (Aberdeen), F.R.C.P., of the Middle Temple, Barrister-at-Law; Dean of the London School of Hygiene and Tropical Medicine. Pp. 206; 6 text illustrations and 72 plates. Baltimore: The Williams & Wilkins Company, 1941. Price, $8.00.

THIS Atlas of Silicosis was written to describe the essential radiologic features of silicosis for senior medical students, industrial physicians, and those dealing with this type of disease.

The author has commented briefly upon etiology, pathology, Roentgen manifestations, and clinical findings in silicosis and some of the nonspecific pneumoconioses. He does not attempt to go into detail concerning many of the aspects of this subject. He has, however, given enough in the first four chapters, which are 30 pages in length, to stimulate one to look elsewhere if interested in this subject. Believing that the average physician is not familiar with the Roentgen findings in a number of these conditions, he has devoted the major portion of his book to illustrating representative chest films with a brief history in both English and French.

The publishers have selected a very good print for this book, but the paper is so glossy that it is exceedingly difficult to read unless light is just right. In selecting a calendar of paper for the illustrations of the roentgenograms of the chest, however, they have done an excellent job. I do not know of any more difficult condition to illustrate than the Roentgen findings in silicosis, and yet in this case the author's attempts have been successful.

E. P.

EPILEPSY AND CEREBRAL LOCALIZATION. A Study of the Mechanism, Treatment and Prevention of Epileptic Seizures. By WILDER PENFIELD, LITT.B., M.D. (Johns Hopkins), D.Sc. (Oxon., Princeton); Professor of Neurology and Neurosurgery, McGill University; Director of the Montreal Neurological Institute, and THEODORE C. ERICKSON, M.A., M.Sc., M.D. (Minnesota), PH.D. (McGill), Associate Professor of Surgery, University of Wisconsin. Chap. XIV, by HERBERT H. JASPER; Chap. XX, by M. R. HARROWER-ERICKSON. Pp. 623; 163 illustrations. Springfield, Ill.: Charles C Thomas, 1941. Price, $8.00.

THE leading contributor of this volume is a widely known surgeon who has done original work in epilepsy. In this encyclopedic work, there is comprehensive discussion of the entire subject concerning epilepsy. Epileptic mechanisms are given careful consideration. The chief surgical contribution is the objective treatment of atrophic cerebral lesions, wherein the surgical technique is minutely described. The cerebral cortex of conscious subjects is observed and the epileptic pattern discharge described. Roentgenology considers the atrophic lesions of the brain and skull. A long chapter is electroencephalography, where it is said to be, "(*a*) an aid to diagnosis of epilepsy and allied conditions, (*b*) a means of classification . . . . , (*c*) a

guide to medical or surgical therapy, (*d*) a means of observing subclinical cerebral disorders, (*e*) an aid to discovery of cerebral disorders allied to those of epilepsy in patients without clinical epilepsy but with migraine, mental disease, behavior difficulties, etc., and, (*f*) an aid in estimating the rôle of heredity in epilepsy." The most useful drugs, phenobarbital, dilantin and the bromides are given careful consideration. Among other subjects, epileptic psychology considers the important psychic seizures. The treatment of acute head injuries is well discussed, and vastly important, is the prevention of post-traumatic epilepsy. This volume is of consequence, more so than any we know of on this subject. As for the publishing, it is a triumph of art. N. Y.

PATHOLOGY OF THE ORAL CAVITY. By LESTER RICHARD CAHN, D.D.S., Associate Professor of Dentistry (Oral Pathology), Columbia University, etc. Pp. 240; 165 illustrations. Baltimore: The Williams & Wilkins Company, 1941. Price, $5.50.

THIS is a text on the special pathology of a selected group of more or less common diseases of the mouth. It is chiefly morphologic, while pathogenesis and etiology are sketchily or slightly mentioned. Some diseases are adequately treated; others are presented in an unsatisfactory fashion. For instance, dental caries is described in less than 4 pages, 2 of which are filled with 4 photomicrographs, while the comparatively rare odontomata and ameloblastomata have been given more than 13 pages, including 15 illustrations. Though the text presents little that would make the book commendable to dentists or dental students, the Reviewer wishes to praise the illustrations, some of which are of superb quality. W. E.

DISEASES OF THE BLOOD and Atlas of Hematology. With Clinical and Hematologic Descriptions of the Blood Diseases Including a Section on Technic and Terminology. By ROY R. KRACKE, M.D., Professor of Bacteriology, Pathology and Laboratory Diagnosis, Emory University School of Medicine; Pathologist to the Emory University Hospital, etc. Pp. 692; 46 text illustrations and 54 color plates. Second Edition, thoroughly revised, reset and enlarged. Philadelphia: J. B. Lippincott Company, 1941. Price, $15.00.

IT is a healthy sign for English-speaking hematology that a book of this size and cost should require a second edition in 4 years. As the first edition was not reviewed in these columns we now present for our readers a lengthier notice than is customary for a second edition.

The book is large because it covers both clinical and laboratory phases of a large and rapidly expanding subject. But it is essentially practical, differing chiefly from Downey's "System" in giving but little space to theoretical considerations. Even such hoary but important controversies as that of the origin of blood cells are presented chiefly on a factual basis, briefly but concisely, as of practical importance to the clinical hematologist. Here, incidentally, the "trialist" theory is offered not because it is the author's preference, but as that most widely accepted. This avoidance of all but the immediately "practical" produces, necessarily perhaps, such regrettable omissions as the valuable non-morphological data on blood cells that have been obtained by the study of living cells (the Lewises, McCutcheon and Coman, and others).

To the contributors to the first edition (J. J. Clark, F. P. Parker, Elizabeth Gambrell—all of Emory University—and R. P. Custer—University of Pennsylvania) has now been added Lloyd Craver, of the Memorial Hospital

of New York. The name of Hortense Garver, co-author of the first edition, no longer appears on the title page.

The book is divided into 8 sections: Hematologic Terminology (40 pages); Development and Morphology of Blood Cells (60 pages); Leukocytosis and Leukopenia (49 pages); the Anemias (193 pages); the Leukemias (66 pages); Hemorrhagic Diseases (34 pages); Miscellaneous Conditions (121 pages); Hematologic Technic (81 pages). Thus, the arrangement is on a "practical" basis rather than in a logical sequence, but well proportioned—the anemias and leukemias occupying more than half the book.

In the first section the pages wisely devoted to terminology permit the student to start from a firm basis. Some novel terms are recommended, preceded by a table of term origins. Most of these (*e. g.*, neutrocytosis, neutropenia, fragilocyte, crenocyte) are distinctly advantageous, to avoid more cumbersome though perhaps etymologically more correct terms. In other cases, as good usage never can be entirely consistent, the older term seems preferable. "Basocyte," for instance, has no advantage over "basophil," which not only has the recommendation of long usage, but also expresses the cell's qualities more accurately; the same with "neutrophil" and "eosino- (acido-)phil."

As far as the Reviewer has been able to determine, all the sections are uniformly good. A pathologist would naturally like more on pathologic anatomy as illuminating the disease processes, but this is hardly to be expected in view of the author's expressed aims.

The illustrations are good (on heavy coated paper) and well explained. Cell magnifications are not given, so that one has to rely on the general statement that they are mostly about 1200 diameters. Anyone desiring but one volume on Hematology would do well to make this the one.

E. K.

EYE HAZARDS IN INDUSTRY. Extent, Cause, and Means of Prevention. By LOUIS RESNICK. Pp. 321; 33 illustrations. New York: Columbia University Press, 1941, for National Society for the Prevention of Blindness. Price, $3.50.

THIS is an excellent book for the layman on the prevention of eye injuries in industry. It is written by a man who has obtained his information first-hand in factories and work shops where ocular injuries are frequent. It does not pretend to be a medical book describing eye injuries, although there is a chapter giving in lay terms the type of injuries and diseases met with. This is perhaps the weakest part of the book.

It is to be recommended to all Social Service workers, hospital authorities, large industrial concerns, and insurance companies, and could be read with profit by every foreman in a factory. F. A.

THE PROCEEDINGS OF THE CHARAKA CLUB. VOLUME X. "Post Multa Virtus Opere Laxare Solet." Pp. 260; illustrated. Baltimore: The Williams & Wilkins Company, 1941, for The Charaka Club. Price, $5.00.

IN the same vein as in previous volumes, this Volume X presents selections from the cultural essays of its members during the past 4 years. Various tastes are served. Some idea of the range covered may be had from such titles as Packard's "Fig Leaves for Shakespeare and Montaigne," Steiner's "Story of Barbara Fritchie," Wortis' "We All Have a Word for It," Whipple's "Ramblings of a Rug Addict," Rake's "An Early Pill Slab and the Lambeth Potters," Lund's essays on Galen and Aulus Gellius. Sachs' "Early Years of the Charaka Club" gives a good picture of this interesting organization. E. K.

DISEASES OF WOMEN. By HARRY STURGEON CROSSEN, M.D., F.A.C.S., Professor Emeritus of Clinical Gynecology, Washington University School of Medicine; Gynecologist to the Barnes, St. Louis Maternity and St. Luke's Hospitals, etc., and ROBERT JAMES CROSSEN, A.B., M.D., Assistant Professor of Clinical Gynecology and Obstetrics, Washington University School of Medicine; Assistant Gynecologist and Obstetrician to the Barnes and the St. Louis Maternity Hospitals, etc. Pp. 948; 1127 illustrations, many in color. Ninth Edition, entirely revised and reset. St. Louis: The C. V. Mosby Company, 1941. Price, $12.50.

THE Ninth Edition of this classic text, "Diseases of Women," had undergone considerable revision to include the advances of this science in the 6 years which have elapsed since the last edition. There have been added 145 illustrations, many in color. No details of operative gynecology are given in this book as they are contained in the companion book on "Operative Gynecology" by the same authors.

Since the major investigative interest in gynecology has shifted in recent years to the physiologic aspect of the subject, it is but natural that a revision should take place, and much new material has been added to the discussion of hormones and endocrine activities. What is useful in these advances has been offered in such a way that the practicing physician may employ them in his daily work. The book will hold the same high mark in gynecological literature attained by its predecessors. P. W.

SEX LIFE IN BABYLONIA. By EDWIN W. HIRSCH, M.D. (From the Urologic and Cutaneous Review, September, 1941.) Pp. 38. Chicago: Research Publications, 1941.

THIS booklet contains matter, reprinted from the Urologic and Cutaneous Review (Sept., 1941), by the author of "The Power to Love" and "Feel Like Thirty at Fifty." It purports to be the first of a series "to help the sexually blind see clearly the issues of life" by studying the sexual life, customs and laws of various peoples. It is written in an unscientific style that includes inaccuracies such as attributing the loss of the Babylonian civilization to the Flood so graphically described in the Bible. Attitudes toward sexual intercourse and demonic influences that were widespread among primitive and early races are naively offered as special qualities of the Babylonians and quite unjustifiable inferences are made to show that they were aware of syphilis, for instance. The surprising opening statements that "more people die from fear than from any other single cause . . . the most deadly form is sexual fear" receive neither explanation nor support in the rest of the text. E. K.

DISEASES OF THE THYROID GLAND. Presenting the Experience of More than Forty Years. By ARTHUR E. HERTZLER, M.D., Surgeon to the Halstead Hospital; Professor of Surgery in the University of Kansas. Pp. 670; 354 illustrations and 2 colored plates. New York: Paul B. Hoeber, Inc., 1941. Price, $8.50.

THROUGHOUT this book there is repeated again and again the following conviction that *total* thyroidectomy should be done for all types of goiters observed after adolescence: ". . . it seemed logical to remove totally goitrous glands in adults, both plus and minus, and the indications, based on experience, are that both states are thus eliminated. In other words myxedema, like hyperthyroidism, is due to some deleterious secretion and not to a cessation of a normal function as commonly believed." As an

example of the literary style, may be cited the following: "It is first of all to be realized that the term myxedema is not synonymous with deficient thyroid secretion. This belief has been one of the sacred cows in medicine and any one who made merry with the old bossie did so at his peril, but the old critter has gone to the hamburger factory for ignominious extinction."

The author intends this to be a personal record of his 40 years' experience in thyroid surgery, and not as a treatise on thyroid disease. Even so, it does not impress the reader that the author has deep knowledge or unbiased good judgment when he does not discuss our present knowledge of iodine metabolism and ignores or ridicules nearly every one's views except his own. There is no bibliography. Many statements on physiology and microscopic pathology are not sound.

The text is not worthy of the very beautiful physical make-up of the book. I. Z.

THE MARCH OF MEDICINE. New York Academy of Medicine Lectures to the Laity, 1941. Pp. 154; 4 illustrations. New York: Columbia University Press, 1941. Price, $2.00.

THE Lectures to the Laity of the New York Academy of Medicine are filling a great need in this day when the laity devour with eagerness every scrap of information on medicine to which they have access. These lectures on very varied medical topics of great importance at the present time, are all delivered by recognized authorities on the subjects which they discuss. While dealing with the deepest scientific material the lecturers present it in a way which not only renders the subject readily comprehensible to a lay audience but excites deep interest in the hearers. F. P.

NEW BOOKS.

The Blood Bank and the Technique and Therapeutics of Transfusions. By ROBERT A. KILDUFFE, A.B., A.M., M.D., F.A.S.C.P., Director, Laboratories, Atlantic City Hospital; City Bacteriologist, Atlantic City; Serologist, Municipal Hospital for Contagious Disease, Atlantic City, etc., and MICHAEL DE BAKEY, B.S., M.D., M.S., F.A.C.S., Assistant Professor of Surgery, School of Medicine, Tulane University of Louisiana; Visiting Surgeon, Charity Hospital, Touro Infirmary, and Mercy Hospital, New Orleans, etc. Pp. 558; 214 illustrations. St. Louis: The C. V. Mosby Company, 1942. Price, $7.50.

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A Hand-Book of Ocular Therapeutics. By SANFORD R. GIFFORD, M.A., M.D., F.A.C.S., Professor of Ophthalmology, Northwestern University Medical School, Chicago; Attending Ophthalmologist, Passavant Hospital, Wesley Memorial Hospital, Cook County Hospital. Pp. 410; 69 illustrations. Third Edition, thoroughly revised. Philadelphia: Lea & Febiger, 1942. Price, $4.00.

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PROGRESS

OF

MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY.

UNDER THE CHARGE OF

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THE PATHOLOGY OF ISLET CELL TUMORS OF THE PANCREAS.

TUMORS of the pancreas possessing a histologic structure closely resembling that of the islets of Langerhans have been recognized for nearly 40 years. Credit for the earliest clear description of an islet cell tumor belongs to Nicholls,[49] who in 1902 reported from this Department an example of "simple adenoma of the pancreas arising from an island of Langerhans." A few reports of similar benign tumors followed in subsequent years, but Shields Warren,[64a] who reviewed the literature in 1926, could find a total of only 16 cases of adenoma of the islets of Langerhans to which he added 4 new cases of his own. Such isolated reports stirred only an academic interest since it was believed, as Warren remarked, that these tumors "probably never give rise to trouble during life and have no clinical significance." With the discovery of insulin in 1922[3] and the recognition of insulin shock,[4,21] there came the realization that oversecretion of insulin might occur and might produce a recognizable clinical syndrome. This idea was crystallized in Seale Harris's concept of "hyperinsulinism."[30] However, the possibility that hyperinsulinism and hypoglycemia might occur as the result of secretory activity on the part of an islet cell tumor was first brought to general attention in 1927 by the report of Wilder, Allan, Power and Robertson[69] of a case in which a carcinoma of the pancreas of islet cell type had produced such effects. Two years later, a case was reported by Howland, Campbell, Maltby and Robinson[32] in which "dysinsulinism" was cured by the surgical removal of an islet cell tumor of the pancreas. Since that time there has been a mounting interest in such tumors and in the clinical syndrome which some of them produce. The total number of reported cases recognized either

clinically or at autopsy now exceeds 100, and successful treatment has become almost commonplace. The clinical aspects of hyperinsulinism and hypoglycemia have been carefully reviewed by Sigwald,[58] Wauchope,[65] Womack,[72] Whipple and Frantz,[68] and others. Whipple and Frantz[68] in 1935, Whipple[67] in 1938, and Frantz[22] in 1940 included in their papers excellent tabulations of the clinical manifestations and pancreatic lesions observed in the cases reported up to the dates of publication. Isaji[34] included a brief résumé of the pathology of islet cell tumors with his report of 2 cases, but so far as the Reviewer is aware, there have been no other attempts to bring together the accumulated information regarding the pathologic features of islet cell tumors of the pancreas.

From the clinical or functional point of view, islet cell tumors fall naturally into two groups: those which produce the hypoglycemic syndrome and those which do not. Anatomic and histologic studies, however, have failed as yet to provide any criteria which permit histologic differentiation of these two groups, so that the pathologist can only classify such tumors as benign or malignant. This distinction has obvious clinical importance, but it has no significance in relation to the possible endocrine activity of these tumors for both the benign and malignant types in some instances produce the hypoglycemic syndrome and in other instances do not. In the absence of clinical evidence of hyperinsulinism or dysinsulinism, islet cell adenomata usually remain unrecognized until discovered at autopsy, while islet cell carcinomata produce a clinical picture which cannot be distinguished from that of any other type of pancreatic carcinoma. Consequently, clinical interest in these tumors depends almost entirely on the ability of some of them to disturb carbohydrate metabolism. Nevertheless, a consideration of islet cell tumors from the pathologic point of view necessitates inclusion of all of them, whether or not they have displayed evidence of functional activity.

For purposes of description and discussion, it is convenient to separate benign and malignant tumors, though it is not an easy separation to make. It is true that some islet cell tumors grow rapidly, invade, metastasize and are obviously malignant. Others are relatively small, grow slowly and lack metastases. However, in certain cases of the latter group the histologic finding of interruption or invasion of the capsule, or even invasion of blood channels by tumor cells, has led those who have reported such cases to doubt that the tumor in question was completely benign. Tumors of this kind have been reported as carcinomata of low grade of malignancy, or as slow-growing carcinomata, or as adenomata, with the question of possible malignancy left in abeyance. Up to the present time the evidence is in favor of the view that these tumors, in spite of their histologic appearance of malignancy, behave as benign growths. In all instances in which an autopsy was obtained, metastases from such tumors were lacking, and in all patients who have survived surgical excision, periodical observation has failed to reveal any sign of recurrence for periods up to 12 years. For the time being, therefore, there is scarcely sufficient reason to create a third category to accommodate these questionable tumors and there is some justification for grouping them tentatively with those which are clearly benign.

Islet Cell Adenoma. *Incidence.* Although Warren[64a] in 1926 found only 16 cases of islet cell adenoma of the pancreas reported in the previous literature, he expressed the opinion that more careful search of the pancreas at autopsy would reveal a higher incidence than that suggested by the then available figures. This view is vindicated by the findings of Pappenheimer[52] who discovered islet cell adenomata in 5 of 4010 consecutive autopsies. In 4 of these cases the pancreatic tumor was an incidental finding and in only 1 had there been clinical symptoms. Probably on the basis of these figures, Campbell, Graham and Robinson[12] stated that the incidence of islet cell adenomata was 1 in 800 or 1000 autopsies but that related hypoglycemic symptoms had been encountered in only 20% of cases. As Whipple[67] has pointed out, "these islet tumors are small, seldom exceeding 1.5 cm. in diameter, and are easily overlooked unless serial sections are made of the pancreas." It is, therefore, entirely possible that the true incidence may exceed even that indicated by Pappenheimer's data. That islet cell adenomata may occur in animals other than man is shown by Hueper's description[33] of an islet adenoma in the pancreas of a white mouse and the report of Slye and Wells[59] of the finding of multiple benign and malignant islet cell tumors in a dog which had shown evidence of hypoglycemia for 2 years before its death.

Taking together all human cases of islet cell adenoma, whether accompanied by clinical symptoms or not, it is found that the incidence is somewhat higher in males than in females. Among 90 reported cases analyzed by the Reviewer, there were 51 males and 39 females. The male preponderance, however, existed entirely among the 64 cases in which there were clinical symptoms of hypoglycemia; of these, 38 were males and 26 females. Among the 26 cases without hypoglycemic symptoms, males and females were equal in number.

Islet cell adenomata have been found in patients of every decade from the first to the ninth. In about one-half of the 90 cases analyzed the patients were between the ages of 30 and 50 years. However, when one separates the cases with hypoglycemic symptoms from those in which such symptoms were lacking, it is found that the lower age groups are filled almost exclusively by patients who suffered from symptoms of hypoglycemia. Twenty of the 90 patients were under 30 years of age and of these only 3 in the third decade showed no clinical evidence of hyperinsulinism. Hypoglycemia due to islet cell adenomata has been reported in children of 7 and 10 years of age,[29,71] but it scarcely ever occurs over the age of 60. One case of hypoglycemia due to islet cell adenoma has been reported in a woman 63 years of age,[34] but the remaining 10 cases of islet cell adenoma in patients over the age of 60 lacked symptoms of hypoglycemia. The oldest patients in this group were 2 males, each 84 years of age.[38,56]

The predominant incidence of the hypoglycemic syndrome in association with islet cell adenomata in the earlier age groups and its virtual absence in the later age periods is probably explained, at least in part, by the fact that the clinical manifestations of hypoglycemia invite surgical exploration of the pancreas with the possibility of early discovery of a tumor; or, treatment failing, the pathologist is likely to be given an early opportunity and the stimulus to search the pancreas carefully at autopsy and perhaps to find an islet cell tumor small enough

to have been overlooked in routine examination. On the other hand, non-functioning islet cell adenomata, even though they may originate in the same age periods, are compatible with survival to a ripe old age.

Gross Pathology. While it is true, as is frequently stated, that the most frequent location of islet cell adenomata is in the tail of the pancreas, the surgeon and pathologist must bear in mind that about 25% of these adenomata occur either in the head or at the junction of the head and body of the pancreas. The remainder are distributed in various parts of the body of the pancreas as well as in the tail. It is also of importance to the surgeon that 2 or more islet cell tumors may be present at different places in the same pancreas. Multiple adenomata have been found in about 12% of cases. In more than one-half of these cases there were 2 adenomata, but as many as 5 were found in a case described by Kalbfleisch,[37] there were no less than 10 in Lloyd's case,[44] and great numbers of small adenomata were distributed throughout the whole pancreas in the cases reported by Lang[42] and Terbrüggen.[61]

Islet cell adenomata occur as circumscribed, spherical, ovoid or irregularly rounded masses of variable size. The smallest have been encountered at autopsy in patients who have shown no clinical evidence of hypoglycemia. Warren[64a] described one adenoma which was only 1.2 mm. in greatest diameter and in his 3 other cases the tumors did not exceed a diameter of 9 mm. However, adenomata of much larger dimensions up to 13 cm. in diameter[15,43] have been discovered at operation or at autopsy in the absence of any clinical record of signs or symptoms of hypoglycemia. On the other hand, the hypoglycemic syndrome has not been observed in association with islet cell adenomata of a diameter less than 1 cm. In the great majority of instances, the tumors have measured between 1 and 2 cm. in diameter[67,68] but there have been reported a few cases of hypoglycemia occurring in association with larger islet cell adenomata measuring up to 11 cm.[51] or even 15 cm.[11] in greatest diameter.

In gross appearance, islet cell adenomata are nearly always described as being sharply circumscribed and discrete, in some instances showing gross encapsulation. In the living patient they present a pink, reddish-gray or cyanotic color contrasting with the paler yellow color of the surrounding pancreatic tissue. The surface of the tumor may be covered by a rich vascular network. These features are well illustrated in the colored plate published by Whipple and Frantz.[68] Some degree of redness or cyanotic discoloration usually persists in the fresh surgical or autopsy specimen and the cut surface of the tumor may show a degree of vascularity evidently greater than that of the surrounding pancreas. The tissue composing the nodule is quite homogeneous in appearance and lacks the lobulation of normal pancreatic tissue.[60] Such tumors are generally somewhat firmer than the pancreatic tissue in which they lie, but they may be soft enough to be difficult to detect by palpation alone when they are buried in the substance of the pancreas.

The progress of fibrosis, hyalinization and finally calcification in some adenomata modifies the color and consistency of these tumors. Depending upon the degree to which these changes have developed there may be a grossly visible increase in connective tissue with a concomitant increase in firmness up to stony hardness. The increase in connective tissue becomes grossly apparent with thickening of the

capsule and the formation of gray trabecular fibrous bands connected with the capsule and traversing the darker tissue of the nodule; or, the tumor may give the impression of complete hyalinization presenting a homogeneous pearly white cut surface resembling that of a hard fibroma. Calcification may or may not be obvious in the gross specimen. The development of marked degenerative changes evidently does not cause cessation of functional activity, for such changes have been repeatedly described in adenomata, the excision of which had effected a clinical cure of the signs and symptoms of hypoglycemia.[34,40,51,68] Moreover, there is no authentic example of spontaneous cure occurring as the result of degenerative changes in an islet cell adenoma. In an interesting case reported by Herrmann and Gius[31] hypoglycemic symptoms were relieved by the excision of a calcified mass embedded in the head of the pancreas. While it seems probable that this mass represented an almost completely calcified islet cell adenoma, no islet tissue could be identified in or around the mass to establish this diagnosis.

Histologic Structure. Unless an extract possessing the properties of insulin is obtained from the tumor, the recognition of an islet cell adenoma depends entirely upon the histologic resemblance of the tumor tissue to normal or hyperplastic islands of Langerhans. Most of the reports of individual cases have been accompanied by adequate descriptions of the histologic structure of the tumor in question, but histopathologic studies as such have been relatively few within recent years, comprising only those of Warren,[64a] Smith and Seibel,[60] O'Leary and Womack,[51] Laidlaw[40] and Isaji.[34]

Warren[64a] laid down histologic criteria for the identification of islet cell adenomata. The first of these was that the morphology and arrangement of the cells must resemble those of the islands of Langerhans. He required also that the mass should measure at least 1 mm. in diameter and that there should be a definite capsule with compression of the adjacent pancreatic tissue. The presence of a definite fibrous capsule is of special importance in distinguishing very small adenomata from hyperplastic islands of Langerhans which may reach a diameter of 1.5 mm. in some cases of diabetes. However, in many islet cell adenomata of larger size the fibrous encapsulation of the nodule is incomplete.[22,40,51,68,73] Compression of the neighboring pancreatic tissue is commonly observed but is not always present.[32,60,68]

Practically all histologic descriptions emphasize the resemblance between the arrangement of the cells in islet cell adenomata and that found in normal islet tissue. O'Leary and Womack[51] and, more recently, Laidlaw[40] have pointed out that there is a tendency in the formation of islet cell adenomata toward repetition on a large scale of the normal structural relations of the islands of Langerhans. The characteristic feature of the normal pattern is the disposition of the cells in short anastomosing cords, 1, 2 or sometimes 3 cells thick, separated by capillary channels with which they are in very intimate relation. The capillaries are extremely thin-walled, being accompanied only by a delicate network of argyrophil reticular fibers and occasional tenuous strands of connective tissue.

This arrangement of the normal island of Langerhans is almost exactly reproduced in some of the islet cell adenomata,[40,51] but in most instances certain features of the normal pattern are exaggerated.

Instead of narrow cords of cells, there may be broad irregular bands many cells thick, or rounded clusters of cells, bounded by thin-walled vascular channels. Most of the islet cell adenomata seem to fall into this general histologic category, showing some fairly narrow cords of cells but with many broader bands and clusters of cells. In other instances the exaggeration of the normal structure is in the opposite direction. Very long narrow cords of cells, only 1 or 2 cells thick, form more or less parallel, undulating and anastomosing bands separated from one another by delicate capillary channels. This "ribbon pattern," as Laidlaw[40] pointed out, is practically identical with that observed in hypertrophy of the islands of Langerhans in cases of diabetes as described by Weichselbaum and Stangl,[66] MacCallum[46] and Cecil.[14] Finally, there is observed in a few islet cell adenomata the formation of small rosette-like clusters of columnar cells radiating from a center at which a minute lumen may be seen. Such structures, too, occasionally occur in normal islets. Laidlaw[40] referred to these as rosettes of cells formed around capillaries, but Isaji[34] interpreted this arrangement as evidence of the formation of short tubules. It is a matter of importance in relation to the genesis of these tumors that true ducts and ductules are often found well within the limits of the tumor mass and that they frequently show continuity with the islet cells of the tumor.[12,22,23,25,32,40,44,51,68,73]

Isaji[34] suggested that islet cell adenomata be classified on the basis of histologic arrangement as "simple solid," "trabecular" (Laidlaw's "ribbon" type), and "partly tubular"; but deviations from the standard patterns render it difficult to apply this classification rigidly. Laidlaw[40] described 2 tumors showing the "ribbon" pattern and regarded this as a most unusual arrangement, stating that he was not aware of any previous description of it. On the other hand, Isaji[34] accepted no less than 9 previously reported adenomata as belonging to this category and stated that these "trabecular" tumors occurred with about the same frequency as the "simple solid" adenomata.

The individual cells composing the islet cell adenomata are also strikingly similar to those of normal islands, especially in the most highly differentiated tumors. In such instances they are of polyhedral, cuboidal, prismatic or columnar shape, depending upon their position in the sinuous cords and lobules. The nuclei occupy a position near the centers of the cells. In those tumors composed of wide bands and clusters, the centrally placed cells are mostly rounded or polyhedral but those which lie next to the vascular partitions are apt to be low or high columnar, forming a peripheral band around each lobule. The nuclei frequently lie toward the capillary poles of these cells. Thus, if there is disintegration of the central cells, the appearance of spaces apparently lined by columnar epithelium may suggest duct formation. In the adenomata of "ribbon" or "trabecular" type, the cells are columnar and lie in a direction transverse to the long axis of the ribbon with both poles of many cells in contact with capillaries. In this case, the nucleus lies near the middle of the cell. Such columnar cells may give the illusion of being abnormally large, but Laidlaw[40] found by actual measurement that they are no larger than many normal islet cells. Nevertheless, the tumor cells are frequently described as being considerably larger than normal.

The nuclei in general are much like those of normal insular cells. They are spherical or slightly ovoid, seldom elongated, varying in size from normal to about one-half again as large as normal. The chromatin content also varies so that the nuclei, though usually vesicular with clearly visible nucleoli, may in some instances appear almost pyknotic. Mitotic figures have been found in varying numbers in some adenomata[22,40,51,68] but in most instances they are absent. The occurrence of binucleated cells and giant cells with central clusters of nuclei has been recorded by O'Leary and Womack,[51] Kalbfleisch[37] and Friedman.[23]

In tissue fixed in any of the ordinary fixing solutions and stained with hematoxylin and eosin or with eosin-methylene blue, the cytoplasm of the tumor cells is found to be definitely acidophilic in contrast to the basophilic cytoplasm of the acinar cells of the pancreas.[32,60,64a] In fresh unfixed and unstained fragments of tissue immersed in Ringer's solution, individual cells can be seen to be packed with minute refractile cytoplasmic granules[13,25,51,73] as are the cells of normal islets.[6a,16,39,50] Careful histologic studies of these specific granules require special tissue fixation and special staining methods. Accordingly, such studies have been carried out chiefly in cases of hypoglycemia in which the pathologist, forewarned, was able to secure prompt fixation of the tissue in appropriate solutions.

The method of Lane,[41] who in 1907 first distinguished between what he named alpha and beta cells, required fixation in 70% alcohol as well as in a chrome sublimate solution. In the former, the granules of the B (majority) cells were dissolved out and the granules of the A (minority) cells could be stained with neutral gentian. With chrome sublimate fixation, the granules of the B but not of the A cells were stained by neutral gentian. Since that time various staining methods have been devised by which the granules of alpha and beta cells are differentially stained in the same section when the tissue is fixed in aqueous solutions so that alcohol fixation is no longer necessary. Nevertheless, O'Leary and Womack[51] have pointed out discrepancies in the results of some of these staining methods and recommended that comparisons which necessitated assigning types to islet cells be made by the original method of Lane. Different investigators have used various types of fixing solutions, including Zenker's stock solution, Zenker-acetic, Zenker-formol (Helly's), Bouin's and Altmann's fluids as well as Bensley's acetic osmic dichromate solution.[51] Fixation in Zenker's, Helly's or Bouin's fluids gives satisfactory results. The special stains most frequently employed are the Mallory-Heidenhain azan stain, and the neutral dyes of Bensley[6a] and Bowie.[9] Laidlaw[40] found the neutral dyes "exasperatingly capricious" when applied to human material, but Bensley's acid fuchsin-methyl green[6a] gave accurate and constant results. Modified fixing solutions and new staining methods have recently been described by Bayley[5] and Gomori.[24a]

By the application of one or other of these special staining methods, it has been shown that a varying proportion of the cells composing islet cell adenomata possess granules which stain like those of normal beta cells.[11–13,23,25,32,40,51,60,64a,68,73] It is thought that the B cells of normal islets are the ones responsible for the elaboration of insulin and it is, therefore, worth noting that such cells have been demonstrated in symptomless adenomata[60,64a] as well as in those with associated hypoglycemia. Studies of adenomata which produced no symptoms and were discovered as incidental findings at autopsy have gone not much farther than this because of the difficulty in demonstrating the specific granules in any but the freshest of autopsy material. In the case of adenomata causing hypoglycemia and excised surgically or obtained at an autopsy

expeditiously performed, the opportunities for histologic study are better and in some such instances cells containing granules like those of A cells have also been described as occurring in varying proportions with those of B type.[12,25,32,40] Laidlaw[40] stated that the tumor cells stain exactly like those of normal islets, most of them containing granules of B type with here and there a cell containing granules of A type. However, as early as 1929, Robinson[32] described certain cells in an islet tumor in which granules of both A and B type were present in the same cell. Prof. R. R. Bensley, whose opinion is quoted by several authors,[13,25,60,62,73] and O'Leary and Womack[51] drew attention to other points of difference between the tumor cells and normal cells of the islets of Langerhans. On the basis of meticulous studies of several different adenomata, Bensley,[25,73] while admitting that the cells bore a considerable resemblance to those of normal islets, a few staining exactly as do normal beta cells, concluded that many of the tumor cells differed from the normal in several important cytologic details. He regarded these as abnormal beta cells. O'Leary and Womack[51] concurred in this view and suggested that the severity of symptoms might be determined by the degree of similitude of the majority of the cells to the B cells of normal islets, as well as by other factors such as the intimacy of relation between the cells and vascular channels, the size of the tumor and the development of degenerative changes. Cells resembling the granule-free C cells of Bensley[6] or the D cells of Bloom[8] are lacking in islet cell adenomata.[40,51]

Hydropic degeneration of the tumor cells such as might suggest excessive secretory activity has not been observed.[40,51] In normal islets other forms of cellular degeneration are rare, but in islet cell adenomata pyknosis of nuclei with diffuse basophilic staining of the cytoplasm is frequently seen in small groups of cells.[51] Moreover, in some of the reported tumors there were focal areas of necrosis occupied by cell débris mixed with red blood cells.[13,25,51,73] O'Leary and Womack[51] were unable to find convincing evidence that these manifestations of degeneration were entirely attributable to poor blood supply since they sometimes occurred in the neighborhood of apparently functioning vessels.

"Just as the tumors duplicate the structure of normal and hypertrophied islets, so they are subject to the same pathological vicissitudes such as fibrosis, hyaline degeneration and calcification" (Laidlaw[40]). Fibrosis begins with an increase of the delicate fibrous framework surrounding the vascular channels and proceeds to the formation of broad bands of collagenous connective tissue which separate the constricted strands and clusters of tumor cells from the blood-vessels. These fibrous bands show a marked tendency to undergo hyalinization and, as elsewhere in the body, the hyaline material is apt to become calcified. Shields Warren[64b] and O'Leary and Womack[51] regard the hyaline material as being derived from the connective tissue and possibly from endothelial cells. Laidlaw,[40] on the contrary, is convinced that degenerating tumor cells also contribute to the formation of the hyaline masses. The extent to which these tumors may be replaced by fibrous, hyaline or calcified tissue has suggested the possibility of spontaneous cure of hypoglycemic symptoms caused by an islet cell adenoma, but no authentic instance of this has been recorded.

Possible Malignancy of Certain Islet Cell Adenomata. As has been mentioned already, certain circumscribed islet cell tumors have been described which lacked metastases and gave no other gross indication of malignancy but which possessed histologic features suggesting this possibility. The problem presented in such instances has recently been discussed by Frantz[22] who collected from the literature the cases of this kind reported up to the end of 1939 and described several additional cases making a total of 21. The histologic findings upon which most emphasis has been placed as indicative of possible malignancy are incompleteness of encapsulation, and invasion of the capsule or of the adjacent pancreas or of blood-vessels by the tumor. Marked variation in size, shape or staining qualities of the tumor cells need not necessarily be an associated finding and mitotic figures may be totally lacking. Indeed, several authors, while making a diagnosis of carcinoma in such cases, have expressed doubt as to its correctness or have at least suggested that the tumor was of low-grade malignancy.[22,25,32,48,51,54,62,73]

Clinical data support the view that these tumors, in spite of their histologic appearance, behave as benign neoplasms. Symptoms of hypoglycemia, evidently indicating the existence of the islet cell tumor, were present in these cases prior to operation or autopsy for periods almost always longer than 1 year up to 5,[25] 7,[32] or 8 years.[54] Yet no metastases were found and surgical excision of the primary tumor in the pancreas was followed by the disappearance of hypoglycemic symptoms. No instance of recurrence of the tumor growth following operative removal has been reported, while survival for periods of 1 year up to 5 years or more without the recurrence of hypoglycemic symptoms has been recorded in 10 cases.[22]

In the case reported in 1929 by Howland, Campbell, Maltby and Robinson,[32] the patient suffered from symptoms of hypoglycemia for 7 years before they were relieved by surgical excision of a tumor diagnosed histologically at the time as a slow-growing carcinoma of the islets of Langerhans. No tumor metastases were found at operation. Dr. W. L. Robinson kindly informed the Reviewer in a personal communication in December, 1941, that this patient was still alive and well, more than 12 years after operation and 19 years after the onset of symptoms attributable to the presence of the tumor. Dr. Robinson stated that in view of this long period of survival and after reviewing the original histologic sections in the light of experience with tumors subsequently studied,[12] he is now of the opinion that the tumor in the first case was not truly malignant. Tempting as it is to do so, the same conclusion cannot yet be drawn with finality concerning all the remaining cases in this group. It is important that these patients should be followed and the ultimate findings recorded for the guidance of both clinician and pathologist.

Associated Changes in Endocrine and Other Organs. Hypertrophy of the islets of Langerhans has been described in a few cases in association with islet cell adenomata of the pancreas,[23,34,37,44,45,61] but this does not occur with any regularity and in most instances the islets are histologically normal.[51,60,64a] There is no histological evidence of excessive secretory activity on the part of the islets and, indeed, direct evidence to the contrary is provided by the disappearance of symptoms of hypoglycemia after surgical excision of an islet cell adenoma.

Among associated lesions in other endocrine organs, various changes in the anterior lobe of the pituitary gland are the most frequent, though by no means common. Diffuse hyperplasia of eosinophil cells,[47] adenomatous hyperplasia of eosinophil and basophil cells,[23] basophil or chromophobe adenomata[23,37,44,47,53,61] and marked invasion of the posterior lobe by basophils[23,53] have been described in individual cases. Adenomata of the thyroid[27,47] and parathyroids[37,44] and enlargement of the thymus gland[37,53,61,75] have also been observed as relatively rare associated lesions. In some instances, several of these lesions have been encountered in the same case together with islet cell adenoma, notably in the cases reported by Lloyd,[44] Rienhoff and Lewis,[53] Kalbfleisch[37] and Friedman.[23] The exact relation between these various changes in endocrine organs is not clear.

The liver is generally found to be essentially normal. Estimations of liver glycogen in several cases with hypoglycemia have shown considerable variations. Small to large amounts of glycogen have been demonstrated in a few instances,[17,45,47,69,75] while in other cases glycogen was virtually absent from the liver.[53,57,61]

In patients with a history of hypoglycemia various microscopic changes have been found in the brain and these have been variously interpreted.[1,23,47,48,61] Perivascular petechial hemorrhages throughout all parts of the brain are common. Nerve cells in all layers of the cerebral cortex, in the basal nuclei, midbrain, pons, medulla and, to a lesser extent, in the cerebellum, are described as showing acute degenerative changes with swelling, chromatolysis and distortion of cell outline. Progression of these changes to complete disintegration of nerve cells with secondary glial proliferation has been described in some instances.[47,48] Such widespread degenerative lesions have generally been regarded as significant, but Baker and Lufkin[1] believed that the acute degenerative changes observed in their cases had occurred postmortem. The petechial hemorrhages have usually been interpreted as the result of convulsive seizures.

Islet Cell Carcinoma. Undeniably malignant islet cell tumors are rare. In only 6 reported cases has the hypoglycemic syndrome been observed in association with such tumors and cases of islet cell carcinoma without symptoms of hypoglycemia appear to be equally rare. However, in the absence of hypoglycemic symptoms, an islet cell carcinoma is less likely to be recognized as such. Peculiar tumors of the pancreas have occasionally been reported which might well have been unrecognized islet cell carcinomata, for example, the diffuse carcinoma of the pancreas of "rare type" with unusual metastases described by Willis.[70] Moreover, at least 1 functioning and 4 non-functioning islet cell carcinomata have been mentioned in passing by different authors without details of the cases being given.[18,24b,44] The 9 cases which have been reported in some detail are summarized in Table 1 and equivalent information is included regarding 3 cases merely mentioned by the Reviewer in a previous publication.[18] The "adenoma malignum" of islet cell type described by Vecchi[63] is omitted since metastases were lacking and there was no other good evidence of malignancy. In addition to its occurrence in man, islet cell carcinoma of the pancreas has been discovered twice in dogs.[10,59]

TABLE 1.—TWELVE CASES OF ISLET CELL CARCINOMA.

TABLE 1.—TWELVE CASES OF ISLET CELL CARCINOMA.

Authors.	Sex.	Age.	Hypoglycemic syndrome.	Total duration of illness (mos.).	Site of primary tumor.	Sites of tumor metastases.
Wilder, Allan, Power and Robertson[69]	M	40	Present	20	Tail of pancreas	Regional lymph nodes, liver, mesentery
Judd, Faust and Dixon[36]	F	18	Present	4½	Whole pancreas	Liver (no autopsy)
Bickel, Mozer and Junet[7]	M	56	Present	20	Body and tail of pancreas	Liver, gall bladder, peritoneum, adrenals, pericardium, myocardium, pleuræ, lungs
Cragg, Power and Lindem[17]	F	41	Present	8½	Whole pancreas	Abdominal lymph nodes, liver
Joachim and Banowitch[35]	F	31	Present	2	Terminal half of pancreas	Regional lymph nodes (no autopsy)
Ballinger[2]	M	53	Present	9	Aberrant pancreatic tissue in liver	Abdom. and mediast. lymph nodes, spleen, adrenals, kidneys, mesentery, lung, myocardium, subcutan. nodule (rt. clavicle)
Zanetti[74]	M	56	Not mentioned	?	Whole pancreas	Regional lymph nodes, peritoneum, stomach, liver
Hamdi[28]	M	52	Not mentioned	?	Tail of pancreas	Liver
Evangelisti[19]	M	65	Not mentioned	?	Body and tail of pancreas	Liver, omentum
Duff[18]	M	32	Absent	3	Whole pancreas	Reg. lymph nodes, liver, peritoneum, pericardium, thyroid, adrenals, renal glomeruli, vertebræ
Duff[18]	M	60	Absent	7	Whole pancreas	Abdom. and mediast. lymph nodes, liver, kidney, myocardium, lung, subcutan. nodule (left clavicle)
Duff[18]	M	45	Absent	5½	Whole pancreas	Abdom., mediast., cervical and axillary lymph nodes, stomach, liver, gall bladder, peritoneum, adrenal, pituitary, kidney, ureter, bladder, lungs, brain

In view of the small number of cases available for analysis, it can only be stated that the sex and age incidence of islet cell carcinoma of the pancreas does not appear to differ materially from that of other types of pancreatic carcinoma.[18] These few cases show a ratio between males and females of 9 to 3, as compared with a ratio of only 4 to 3 among cases of islet cell adenoma (including those suspected of being malignant). It is perhaps worthy of note that the very young and the very old are not represented in this group as they are among the cases of islet cell adenoma.

The clinical signs and symptoms in 6 cases were predominantly those of hypoglycemia; the malignant neoplasm as such scarcely influenced the clinical picture. On the other hand, in at least 3 cases there was no clinical evidence of hypoglycemia and the clinical course did not differ from that observed in cases of carcinoma of the pancreas of other histologic types. Regardless of the character of the clinical picture, the duration of illness, from the onset of symptoms to death, was longer than 1 year in only 2 cases and in the majority it was much less than a year. This is in marked contrast to the long periods of illness prior to operation and the even longer periods of survival in good health following excision of circumscribed islet cell tumors in those cases in which the tumors were suspected of being malignant on histologic grounds.

The fact that the islet cell carcinomata in the 12 tabulated cases occupied either the tail, or the tail and the body, or the tail, body and head of the pancreas recalls the greater frequency of occurrence of islet cell adenomata in the tail of the pancreas, as compared with the body and in the body as compared with the head. The part of the pancreas occupied by the carcinoma is usually described as being somewhat enlarged and firm, rubbery or even stony hard. If the surface of the pancreas is not obscured by fusion with neighboring structures, it is found to be gray and fibrous in appearance with smoothly rounded contours interrupted in some cases by nodular projections of yellowish-gray tissue. The cut surface reveals replacement of the normal pancreatic lobules by broad areas of firm, gray, coarsely lobulated tissue, resembling fibrous tissue, which merges imperceptibly into the adjacent intact pancreas. In some instances such tissue may be seen, even at some distance from the main tumor mass, spreading out between the pancreatic lobules and blurring their outlines. In about one-half of the cases, distinctly yellow or reddish-yellow nodules, from a few millimeters to 1.5 cm. in diameter, are found embedded in the gray tumor tissue. Such masses may produce a nodular irregularity of the external surface of the pancreas. While the tumor mass may, in some instances, be fairly sharply demarcated from the neighboring pancreatic tissue, gross encapsulation of the mass has never been described. Metastatic tumor nodules may be circumscribed or infiltrating; the tissue is firm but, like that of the primary tumor, varies in color from gray to reddish-yellow.

The resemblance between the histologic structure of these tumors and that of normal islets of Langerhans has been repeatedly commented upon. This similarity is most striking in small outlying nests of cells which may be extraordinarily difficult to distinguish from normal islets.[17] Even in the midst of the tumor mass, rounded structures may be encountered that look like giant islands occurring in masses, some of which are large enough to be grossly visible.[74] Apart from such circum-

scribed structures, however, the tumor takes a diffuse infiltrating form with the tumor cells arranged in rounded clusters or irregular cords separated either by delicate strands of connective tissue carrying numerous capillaries or by broad dense fibrous bands. The clusters and cords of cells are solid and there is no tendency toward the formation of glandular structures or ducts but progressive degeneration and necrosis may lead to the development of cystlike spaces.[7]

The smaller tumor cells resemble normal islet cells very closely, possessing a vesicular nucleus with a distinct nucleolus and eosinophilic granular cytoplasm. In a few cases, the cells were uniformly of this small type,[17,19,28] but considerable variation in size and shape of the cells is the rule. Cells somewhat larger than normal frequently contain large hyperchromatic nuclei and scanty cytoplasm. Multinucleated giant cells have been described in 2 cases.[2,7] Mitotic figures, some of them atypical, are generally present, though in varying numbers. The histologic structure of the metastases is similar to that of the primary tumor, but in several instances there have been observed histologic differences of such a character as to suggest more rapid growth in the metastases than in the primary pancreatic tumor.

The demonstration of specific cytoplasmic granules in the tumor cells has been attempted in only 1 of the tabulated cases and in 1 other. Joachim and Banowitch[35] were able to find a few granules which stained like those of B cells, but Gomori[24b] stated that the tumor cells in his case contained no identifiable granules.

From all of the available information, it would appear that islet cell carcinoma of the pancreas is a rather rapidly growing tumor which spreads quickly from the pancreas into neighboring structures and metastasizes widely by way of both lymphatic and blood channels. The tendency of the tumor to spread rapidly beyond the limits of the pancreas might be regarded as a property inherent in this particular tumor were it not known that the same tendency is shared in equally marked degree by pancreatic carcinomata of other histologic types when they are located in the body or tail of the pancreas. This fact has been pointed out by the Reviewer in a previous publication and the phenomenon explained on purely anatomic grounds.[18]

Genesis of Islet Cell Tumors. The studies of Bensley,[6] Grauer[26] and others on the embryology and regeneration of the pancreas have firmly established the view, now universally accepted, that the epithelial cells of the islets of Langerhans are derived by differentiation from the epithelium of pancreatic ductules. In the embryo, the pancreas develops from hollow epithelial buds which grow out from the duodenum as branching pancreatic ducts. At intervals along these proliferating ducts, differentiation of the epithelium occurs in two distinct directions giving rise either to acinar cells or to islet cells. This ability of the duct epithelium not only to form new ducts but to differentiate into acinar or islet epithelium is certainly carried beyond the period of embryonic development and apparently persists throughout life. In adult animals, ligature of the pancreatic duct is followed in a short time by degeneration of the pancreatic acini and by a more gradual disappearance of the islets of Langerhans. So long as the duct remains occluded, attempts at the regeneration of pancreatic acini are constantly frustrated by degeneration of acinar cells as quickly as they are

formed.[6] However, the reëstablishment of duct drainage is followed by rapid and successful regeneration of acini from the duct epithelium.[26] On the other hand, even if occlusion of the pancreatic duct is permanent, the islets of Langerhans are replaced, almost as rapidly as they are destroyed, by new islets originating by differentiation from epithelial cells of the pancreatic ductules.[6] Once differentiated out from the duct epithelium, the islet cells are capable of independent proliferation.

On the basis of this knowledge, it has been suggested by O'Leary and Womack[51] and Laidlaw[40] that islet cell tumors represent a response of pancreatic duct epithelium to a stimulus which calls into action its islet-forming potentiality and, to a lesser extent, its ability to build ducts, while its power to form pancreatic acini remains virtually in abeyance. This suggestion is supported by the frequent presence in islet cell adenomata of pancreatic ducts and ductules[12,22,23,25,32,40,44,51,68,73] in such numbers that at least some of them must be regarded as newly formed.[40] Still stronger support comes from the observation of direct continuity between the epithelium of these ductules and the islet cells of the tumor.[23,25,40,51,73] O'Leary and Womack[51] have even identified single islet cells interspersed among the cells lining the ducts. Moreover, they repeatedly noted the presence of mitotic figures in islet cells containing specific granules, indicating the ability of these cells to carry on independent proliferation once they have differentiated out from the duct epithelium. Although these observations have been made exclusively in islet cell adenomata, it seems justifiable to apply the obvious conclusions to the genesis of malignant islet cell tumors as well.

The ability of the multipotent pancreatic duct epithelium to give origin to islet cell tumors in the pancreas must surely be duplicated in aberrant pancreatic tissue which is usually composed of pancreatic ducts and acini, often with well-formed islets of Langerhans. Pancreatic tumors of other kinds certainly may occur, as exemplified by the adenoma of duct or acinar type discovered by Schmidt[55] in a nodule of accessory pancreatic tissue in the wall of the jejunum. However, so far as the Reviewer is aware, there have been only two reports of the occurrence of islet cell tumors in aberrant pancreatic tissue. Vecchi,[63] some years ago, described a large islet cell adenoma which had developed in accessory pancreatic tissue located near the tail of the pancreas. The second instance is that recently reported by Ballinger[2] in which hypoglycemia was associated with an islet cell carcinoma originating in aberrant pancreatic tissue in the liver (see Table 1). Faust and Mudgett[20] have recently collected from the literature 370 cases of aberrant pancreas. Their analysis of the distribution of the aberrant tissue shows that it was located most frequently in the wall of the duodenum (30%), stomach (26%), jejunum (18%) and ileum (13%); other rarer sites included the gall bladder, spleen, mesentery and omentum. It seems obvious that islet cell tumors may be expected to occur rarely in one or other of these situations.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY.

UNDER THE CHARGE OF

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FAMILIAL AGGREGATION IN POLIOMYELITIS.

THERE are epidemiologic indications that the development of paralytic poliomyelitis in the few of the many who come in contact with the virus may be determined not so much by parasitic or environmental factors of exposure, but rather by autarceologic susceptibility of the host.[1b,c] In a smaller proportion of cases, certain temporary or changing influences[1h,i,4,6,8,9,22] in the individual, operative at the time of development of the disease, clearly affect the outcome of exposure to the virus. But a number of selectivities in the occurrence of the frank disease among those exposed indicate that susceptibility to paralysis is even more often inherent.[1e–f]

It should be clearly in mind that susceptibility to paralysis must await exposure to the virus for its expression. Otherwise, confusion arises when an attempt is made to reconcile the distribution of the frank disease with the idea of susceptibility as an important determinant of paralysis. Thus, an eminent epidemiologist once rejected the whole idea of individual susceptibility to paralysis on the grounds that such a susceptibility could not by any stretch of the imagination occur radially (in the manner of spread of epidemics of the disease).

It is recognized, of course, that the development of poliomyelitis is immediately dependent on exposure to the virus and that many of the features of the distribution of the disease tend rather to reflect the distribution of the virus. For example, it is now clear that the age distribution of the disease is determined by the time (or average age) of exposure to the virus and may be limited by immunity from previous exposure, rather than by variations in susceptibility with age *per se*.[1a,3a–c,10,26]

What is determined by inherent or natural immunity (autarcesis) is the limited and selective occurrence of paralytic poliomyelitis in those exposed to its virus. It is this aspect of the disease that takes away from the presenting epidemiologic picture the readily apparent criterion usually sought to establish contagion—the occurrence of the disease among those directly associated with the sick—and which accounts for the ease with which the whole subject of transmission can be reopened by the advancement of any new hypothesis, based on even fragmentary observations on some feature of the distribution of the more apparent clinical disease when not viewed in the light of the less apparent but much more widespread dissemination of the virus.

Because of the indirect nature of much of the evidence for it, the epidemiologic concept of widespread dissemination of virus originating in the now classic observations of Caverly and Wickman has been subjected to many checks and challenges. The basic evidence lies in the unbroken gradation from frank paralytic cases of the disease through milder and non-paralytic forms to suspected abortive infections; in the

time and space relationships between clinically recognizable cases which imply intervening milder infections or healthy carriers; and in the occurrence of extensive immunity in the general population as shown by virus neutralization tests.[3a–c] The concept of widespread virus is at the present time being rapidly substantiated by new procedures for the detection of virus, not only in recognizable cases but in suspected illnesses and healthy individuals as well.[15,20] Only when the distribution of the frank disease is examined in the light of widespread virus does it become possible to discern the selectivities which indicate inherent or familial susceptibility to paralysis as the factor which determines this complication in the few of the many exposed. The pattern of paralytic disease occurrence is formed by interceptions of the pattern of susceptibility to paralysis by that of virus distribution.

Doubtless much of the confusion which has entered into the formulation of a generally acceptable concept of the natural history of poliomyelitis has arisen from the difficulties in establishing either of the two variables which determine the pattern of disease distribution, when this resultant pattern has been the one directly presenting itself for study. Now that one of these determining patterns can be more or less held fixed as a variable, the other can be brought nearer the surface for examination.

In this paper are reviewed several sets of observations,[1j] all of which, under the concept outlined, are consistent with hereditary susceptibility as a factor which determines the occurrence of paralysis in a selected few of the many exposed to the virus. The data do not represent a systematic tabulation of the different categories of cases as to frequency of occurrence in a given population or series of cases. They have been accumulated over a period of years in the course of various studies in numerous localities, and hence no comparison can be made with any calculated expectancy. For these reasons, no one of the group of instances can be considered in itself proof of the operation of hereditary factors in the distribution of paralytic poliomyelitis. But from the rareness with which some of the instances would be expected to occur by chance in the distribution of a relatively uncommon disease, the observed frequencies are suggestive of hereditary factors in the distribution of paralytic poliomyelitis. Furthermore, the different categories of instances occur in general in the proportion to each other which would be expected in a disease influenced by heredity. Although the nature of the data available precludes exact comparisons, there are certain indications that the frequency of the different categories of instances is of the same order as is observed in corresponding instances of certain other diseases in which hereditary influences operate.[1g,13,14] Family composition itself, of course, is one factor in determining the relative proportions of some of these instances and would be a more decided determining factor, for example, between one disease preponderantly of childhood and another preponderantly of adult life. Because of the more or less general agreement between the frequency of these instances in poliomyelitis and in diabetes (a non-infectious disease occurring with a very different age distribution), to be shown later, we are inclined to the view that the relative frequencies of the different instances recorded here are indicative of hereditary factors influencing the distribution of poliomyelitis.

Multiple Cases in Families. The first of these observations is a reinterpretation of certain data relating to the frequency of multiple cases of poliomyelitis in families. One of the commonplace arguments against the widespread and easy transmission of the virus from person to person has been the infrequency of multiple cases in families, which has been commonly interpreted as evidence that the virus of poliomyelitis is less communicable than those of other common infectious diseases which tend to spread through families. This idea was emphasized in an early study by Hill[11] in Minnesota, where he "compared the relative transmissibility of infantile paralysis with that of scarlet fever, diphtheria and typhoid fever in the same territory." Of persons known to have been exposed to diphtheria, scarlet fever and infantile paralysis, the percentages contracting the disease were: scarlet fever, 22%; diphtheria, 17%; infantile paralysis, 6%. Taking the instances of these diseases where 1 case existed in a family, other cases occurring in the family were as follows: scarlet fever, 40%; typhoid fever, 30%; diphtheria, 29%; infantile paralysis, 17%. From these observations, Hill concluded that poliomyelitis is less transmissible than scarlet fever, typhoid fever, or diphtheria. From these and similar findings, it was concluded by certain students, many years ago, that "such instances are difficult to explain by any theory of personal contact, direct or indirect."[18]

Lavinder, Freeman and Frost,[17] from detailed comparisons of secondary attack rates in poliomyelitis, scarlet fever and diphtheria, concluded that secondary attack rates indicate quite striking differences in the liability of persons exposed in the family with a case of the disease to contract it. In poliomyelitis, only 1 person in 100 exposed in the family with a case developed the disease, while in scarlet fever the proportion was 1 in 10, and in diphtheria 1 in 16 in those so exposed.

On the other hand, the striking manner in which poliomyelitis sometimes runs through several members of a family under the same conditions of exposure where only 1 member is usually attacked has long suggested a family tendency.[25] Marie[19] and also Charcot, of the early French writers, laid great stress on the hereditary nature of the disease, pointing to its known occurrence in several members of the same family as evidence of a hereditary influence. Stephens,[23] reporting an outbreak in Victoria, Australia, in 1908, makes the statement that there is a family predisposition to the disease in some instances.

In 1925, Aycock and Eaton,[2] in the course of a study of the interval between multiple cases of poliomyelitis in families, made some calculations (unpublished) on the probability of the occurrence of additional cases in a family into which the disease was introduced, and found that the attack rates in remaining members increased with the number of cases which had occurred. Because of the nature of the data and the methods of calculation, no conclusion could be drawn, but a family tendency was suggested.

Of these two contrasting points of view of the contagiousness of the virus of poliomyelitis, the latter seems preferable, since the observation made by Hill to the effect that poliomyelitis is less contagious than diphtheria and scarlet fever, and those by Lavinder, Freeman and Frost, generally interpreted in the same way, do not include adjustment for differences which would be expected in familial aggregation in relatively

rare and relatively common diseases in the general population. Now, if population saturation in poliomyelitis is equal to that of measles and diphtheria, for example, then poliomyelitis virus infection (not clinical disease) must be as contagious as those of measles and diphtheria, and there is no reason for supposing that any special condition of contagion would cause the spread of the virus of poliomyelitis, upon introduction into a family, to depart markedly from that of measles in a family. As is well known, measles tends to run through families, and as indicated by the weight of evidence in general and recently verified by virus detection studies such as that of Langmuir and McClure,[16] the same holds true of poliomyelitis virus. Since measles is always clinical, the observance of cases in non-immunized members of families is a true measure of virus spread. But since poliomyelitis virus infection is silent in the vast majority, the same silence would be expected in families. Stated in another way, if the occurrence of secondary cases of measles in a family can be considered as reflecting the contagiousness of this virus in general, it would be expected—bearing in mind the relative infrequency of clinical poliomyelitis—that the occurrence of secondary cases of poliomyelitis in families, reflecting only in a small degree the contagiousness of this virus, should be correspondingly less. Since measles uniformly occurs in clinical form and clinical poliomyelitis occurs in less than 1 % of those exposed to its virus, it would be expected that the tendency to secondary cases of poliomyelitis in families would be of the order of 1 % of that observed in measles. Thus, the finding of a secondary attack rate in poliomyelitis, as in the studies of Hill and of Lavinder, Freeman and Frost, as high as one-fifth that of measles (Chapin[7]) is in reality indicative of a relatively high tendency to familial concentration of clinical poliomyelitis.

Recurrence in Siblings. Because of the relatively low incidence of poliomyelitis—the infrequency of outbreaks in an average community—recurrences in the same family would on the laws of chance be exceptional. During the course of observations covering a period of years, instances of poliomyelitis have been encountered in a brother or sister of some patient seen in previous years, or where some older brother or sister of the patient had residual paralysis from an earlier attack. Twenty-two families where dates and ages are known have been studied in detail.[1c] Chronic carriage of the virus on the part of the earlier case, or some household circumstance might be considered as the epidemiologic factor in the recurrence of the disease in the same immediate family. However, it was found that many of the later cases were not yet born when the earlier case occurred, and in several instances the older member of the family was no longer in the home when the later case occurred. Moreover, a number of these cases developed after the individuals had left home, or the families had moved to new localities or even to other countries in the interim between cases. There is one such instance in a family in which both the parents died while the children were infants. The two children were adopted in different localities and never saw each other again, yet each developed the disease at different places years after they had separated.

Due to the wide range of variation in the occurrence of the disease within the period and between the localities represented by these instances of recurrence, any calculated expectancy for statistical com-

parison with these observations would probably come no nearer answering the question than the impressiveness of the observation in view of the uncommonness of the disease in a given location.

Recurrence in Parent and Child. Another circumstance of familial recurrence of poliomyelitis to be considered in the present connection is the appearance of the disease in a child whose parent also had the disease in childhood. Such instances are more suggestive of a hereditary factor than recurrence in siblings, first because they include successive generations; second, because environmental or household influences which might enter into recurrence in siblings can be more definitely excluded; and, third, because the low incidence of the disease would render unlikely the occurrence on the laws of chance of any number of such instances.

Again, no useful comparison can be made with any calculated expectancy, but the number of instances of this type of recurrence appearing in the records of poliomyelitis in Vermont and Massachusetts (with some additions from studies made elsewhere by the author) are regarded as suggestive, when compared with the number of instances of the disease occurring at the same time in a parent and one or more children.

There are in our data 11 instances of simultaneous poliomyelitis in parent and child, which represent a fairly complete reporting of such cases actually occurring in the series from which they were taken, for the simple reason that the recognition of one case practically insured finding the other. On the other hand, the 38 instances of the occurrence of the disease in the offspring of individuals who had had it in childhood doubtless represent only partially the instances which actually exist in the series. These instances were not sought for systematically, and it was only in the course of other investigations that many times they came to light through finding a parent with a limp characteristic of poliomyelitis in whom such a diagnosis had never been made. It is felt that these instances of poliomyelitis in parent and child actually are of more frequent occurrence than is represented by the number we have on record.

Occurrence in Other Relatives. If recurrences of poliomyelitis in siblings and in parents and children are indicative of a familial tendency, recurrences in other relatives in both lineal and collateral lines should also be found. A study in Massachusetts and Vermont of 586 cases of poliomyelitis investigated serially with varying degrees of thoroughness revealed that 26.8% of them had one or more relatives with the disease other than members of their own household. In Massachusetts, 17.7% of the serial cases investigated gave a history of the disease in relatives, in Vermont 51.5%. In 133 family lines in Massachusetts with more than 1 case, the average occurrence was 2.8 cases per family; while in the 89 Vermont family lines with more than 1 case, the average was 3.2 cases per family. Several factors seemed to enter into the higher figure obtained in Vermont. First, many families still living in the old home with members of older generations were able in general to furnish more complete genealogies and had a more intimate acquaintance with their relatives and a better knowledge of their diseases than had residents of cities who were interviewed and who were out of touch with their relatives. Third, in certain local rural outbreaks, a large proportion of cases were observed in closely related individuals in the com-

munity. In one instance especially, doubtless due to the fact that the place had previously been inaccessible to transportation facilities for a good part of the year, a large proportion of the cases of the disease over a period of years occurred in a group of relatives in whom intermarriage was common. In some of these families, subsequent follow-up has revealed as many as 20 cases in the blood relatives of a patient.

In the course of this study in Massachusetts and Vermont, 100 individuals who had not had poliomyelitis themselves, nor had it occurred in their siblings, were chosen at random as a control. Only 5% of this group could cite single cases among their relatives.

Since New England is an area of comparatively high poliomyelitis prevalence, the familial occurrence of the disease in a southern state, where the reported prevalence is low, has been studied with similar results.[1d]

Twins. Data concerning the occurrence of disease in twins have many times been used in studies of inheritance. Much can be learned from a comparative analysis of the incidence of a disease in similar and dissimilar twins, for if a condition is inherited, its incidence in both similar twins should far exceed its incidence in dissimilar twins. In Joslin's[14] series of patients with diabetes (about 17,000), 48 pairs of twins suitable for analysis were found. Of these twins, 19 were similar in type, 29 dissimilar. In 12 of the 19 sets of similar twins, both were diabetic (63%), whereas in only 2 (7%) of the 29 pairs of dissimilar twins did both twins have the disease.

Our poliomyelitis data include 9 sets of twins, of which 4 sets are known not to be identical, where 1 developed paralytic poliomyelitis; 1 set (identical) where 1 developed paralytic and the other non-paralytic poliomyelitis; 1 set (not identical) where both developed paralytic poliomyelitis; 1 set (girls) who both had non-paralytic poliomyelitis; and 1 set (identical) who both developed paralytic poliomyelitis. In the 4 instances where both twins developed the disease, it occurred at the same time.

Double First Cousins. In the study of poliomyelitis in the relatives of 586 patients (with incidental additions from time to time as family histories have become available), exact relationships have been charted. On these charts there are 97 instances where 2 or more first cousins have had the disease, and 3 instances of double first cousins. In 1 of these, 2 brothers who had a first cousin with poliomyelitis married 2 sisters who had an aunt, a first cousin (daughter of the aunt), and 6 other blood relatives with poliomyelitis. Two children of 1 of these marriages had poliomyelitis in 1916, and 1 child of the other marriage, living in the same locality but not born until 1919, had poliomyelitis in 1921.

There are two additional instances of double first cousins where 1 had poliomyelitis and the disease occurred in the children of the other; and 2 instances where the disease is known in the grandchildren and great-grandchildren of double first cousins. In all of these instances, the disease is known in 1 or more other blood relatives.

Also included in this category is an instance where a son by a prior marriage and his father, with 1 other case in a relative, married 2 sisters in whose relatives 9 cases were traced. A child of the son and a grandson of the father by the second marriage developed poliomyelitis in the same city, 1 in 1916 and 1 in 1930.

Husband and Wife. From the fact that poliomyelitis is preponderantly a disease of younger years, it would be expected that more than 1 case in a family or household at adult age, as for example in husband and wife, would be exceptional. However, a number of instances do appear in our data in which the disease has occurred at adult age in 2 members of a family. The familial significance of these multiple adult cases lies in the fact that they are blood relatives—2 cases in adult siblings, and 1 particularly illustrative instance where 3 cases occurred at the same time—in a child, his father and an adult sister of the father; the wife in the household escaping.**

TABLE 1.—OCCURRENCE OF POLIOMYELITIS, DIABETES AND LEPROSY IN RELATIVES OF PATIENTS.[J]

	Poliomyelitis.	Diabetes.	Leprosy.
Consecutive cases studied	586+*	523	100
Controls	100	153	
Patients with cases:			
In siblings	109	100	28
In parents	48	86	19
In other relatives	294	110	12
Total patients with cases in all relatives†	314	296	59
Total controls with cases in all relatives	5	26	
Instances in twins:			
One or both	9	48‡	
Both	3	14	
Identical twins:			
One or both	..	19	
Both	..	12	
Non-identical twins:			
One or both	..	29	
Both	1	2	
Instances in husband and wife	1	22§	5¶
In related husband and wife	1	1§	Yes¶

* Because of a number of later additions and overlapping due to more extended studies of some of the original cases, the exact number of cases cannot be stated.

† Patients are counted more than once if cases occurred in more than one category of relatives.

‡ From records of about 17,000 cases.

§ From records of about 1000 cases. (We are indebted to Dr. Priscilla White for these figures.) No special attempt was made to ascertain relationships between husbands and wives with diabetes.

¶ In the study by Hopkins and Denney[12] it is not stated whether conjugal cases are blood relatives, nor is it clear that the development of the disease was "conjugal" (marriage between patients already having leprosy are known); but in other studies (Rogers and Muir[21]), conjugal leprosy is exceptional. In the few instances of conjugal leprosy investigated by the author of the present paper, the conjugal mates have been blood relatives.

There are far more families where the 2 adult members are husband and wife (most often unrelated) than where the adults are blood relatives. In view of this circumstance, the striking rarity of conjugal

** Of interest in this connection, we find in the series of instances of poliomyelitis in parent and child 11 families with 20 cases in the relatives of the parent who had the disease, and only 4 families with 11 cases in the family of the parent who did not have the disease. In 3 families, there were other cases in the family of the parent who had the disease, as well as in the family of the one who did not have the disease; but in some there were interrelationships.

poliomyelitis (a phenomenon well known in leprosy) is in itself strongly suggestive of familial predilection. Moreover, in the 1 instance of simultaneous occurrence of the disease in husband and wife (in our records), which is discussed in the section on rural outbreaks, they were blood relatives.

Comparison With Other Diseases. As previously stated, the frequency with which the different categories of familial aggregation in poliomyelitis appear in this series is of the same general order as that observed for corresponding instances of other diseases in which hereditary influences operate. Available for comparison are the extensive studies of Joslin and his coworkers[14] on diabetes and similar studies on the familial occurrence of leprosy by Hopkins and Denney.[13] Corresponding figures for the three diseases are not always available and, due to differences in the methods of collection and analysis of data in the different series as well as certain discrepancies in the tabulations concerning poliomyelitis, exact comparisons are not always possible. Nevertheless, the relative frequency of the various categories of familial aggregation in three diseases, so widely different from an epidemiologic point of view, may be seen from the figures which have been assembled in Table 1 to be roughly comparable.

Other Circumstances Suggestive of Familial Predilection. Other observations, which do not lend themselves to any statistical analysis but which are equally impressive to the investigator in the present connection, are the finding of blood relationships between cases in localized outbreaks and cases which have been encountered consecutively in the course of various studies.

Successive Cases. About 10 years ago, while on a few days' visit to my home town in Georgia, a local physician asked me to see a child he "thought" had poliomyelitis, although he had never encountered a case in his practice in the town. The child had paralysis of the muscles of 1 leg and the diagnosis was verified. The mother of the patient, whom I had known since childhood, had always been lame with what I supposed was "white swelling." The father was also lame as a result of an injury. At the time, it seemed only oddly coincident that all 3 members of 1 family should be lame in the right leg—1 from tuberculosis of the hip, 1 from poliomyelitis, and 1 from an injury.

A few years later, when I became interested in the familial incidence of poliomyelitis, it occurred to me that the mother might actually have had poliomyelitis. On a subsequent visit, I was able to make a positive diagnosis by examination, and obtain the story that, in 1889, when Mrs. P. was about 5 years old, she had an illness which the doctor called typhoid fever, with the explanation that after a few days the fever settled in the leg and withered it.

Another of the few cases in this small town, where poliomyelitis has been rare, occurred the same year. In my childhood, there had been a colored boy who went about the streets in a goatcart, with atrophied, contracted and deformed extremities—poliomyelitis undoubtedly to me now. I located his father, Uncle Dad Martin, still living and over 85, he thought. He could give but meager information, but after some difficulty in establishing dates the story pieced together was that the boy had been well until he was about 5 years of age, in 1889. He was suddenly seized and, as the old man expressed it, "He jes got stiff, and

den he got limber, and nevah did walk no mo'," not too inaccurate a description of poliomyelitis obtained 50 years later, of rigidity of the spine, then flaccid paralysis and permanent disability.

In 1935, in Asheville, N. C., for a day seeing an acute case of poliomyelitis with members of the city health department: a sister of the patient came into the room with a gait typical of poliomyelitis, although the only diagnosis which had ever been made was that, as a small child, she had slipped on a rug and had been lame ever since. The family came from an adjacent rural county. Six months later, when passing through Asheville again, I encountered on the street a young man who was obviously an old poliomyelitic. The diagnosis was verified by examination. His name was the same as the patients of 6 months before, he had come from the same rural section, and was a relative.

In Raleigh, N. C., during an outbreak of poliomyelitis, two physicians in charge of a children's institution, in the course of a discussion of precautionary measures, both happened to mention that poliomyelitis

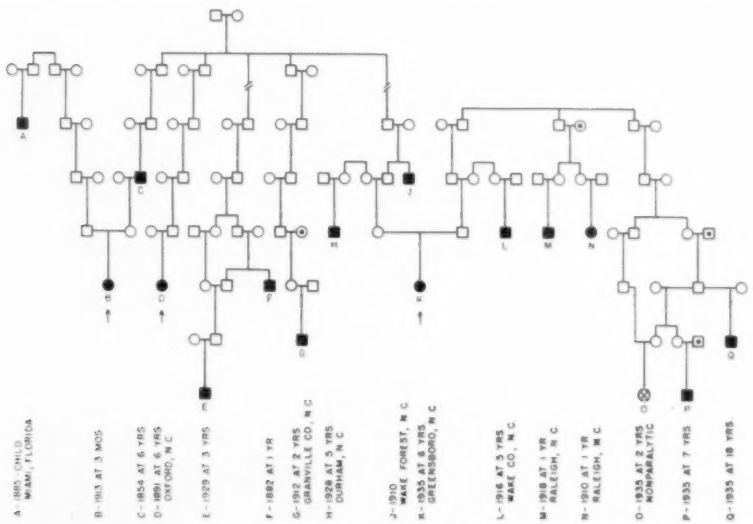
A - 1885 - CHILD
MIAMI, FLORIDA
B - 1913 AT 3 MOS
C - 1854 AT 6 YRS
D - 1891 AT 6 YRS
OXFORD, N C
E - 1929 AT 3 YRS
F - 1882 AT 1 YR
G - 1912 AT 2 YRS
GRANVILLE CO., N C
H - 1928 AT 5 YRS
DURHAM, N C
J - 1910
WAKE FOREST, N C
K - 1935 AT 6 YRS
GREENSBORO, N C
L - 1916 AT 5 YRS
WAKE CO., N C
M - 1918 AT 1 YR
RALEIGH, N C
N - 1910 AT 1 YR
RALEIGH, N C
O - 1935 AT 2 YRS
NONPARALYTIC
P - 1935 AT 7 YRS
Q - 1935 AT 10 YRS

CHART 1.—Poliomyelitis. Three cases indicated by arrows were encountered consecutively. Their relationships to each other and to other cases as revealed by subsequent investigation are indicated. Some of the cases occurring in earlier years were verified by examination, others are included as cases of poliomyelitis because of histories from which a diagnosis could be made with at least reasonable certainty.

had occurred in their families, 1 in a daughter and the other in a sister. Their first response to the suggestion that they were related was that there was no kinship, but soon it developed that the mother of the second patient was related to the first patient.

The next day in Greensboro, N. C., seeing an acute case, I learned that the child's mother came from the same community as the two physicians I had met in Raleigh and was related to their families. Upon further investigation, 13 other cases of the disease were found among their relatives. These relationships are shown in Chart 1.

Isolated Rural Outbreaks. In the epoch preceding the era of bacteriologic causation in disease, the prevailing view of leprosy, originating in studies of familial aggregation by Danielssen and Boeck in 1848, in an isolated and sparse focus of the disease in Norway, was that the disease was hereditary. The discovery of the Hansen bacillus in 1874 "threw a flood of light on the etiology of leprosy and revolutionized our entire conception of its epidemiology by displacing the then dominant and paralyzing hereditary theory of its origin by the now generally accepted and more hopeful infective one" (Rogers and Muir[21]).

These divergent concepts—heredity and contagion—derived at different periods, not so much from conflicting observations as from resisting inferences evolved from the same kind of observations, were formed long in advance of precise knowledge of the fields into which they led. Turning back to the data from which these earlier concepts evolved—each doubtless influenced at the time by the ascendency of the field concerned—discrepancies in each and validities in both become apparent. The approval of either necessitates the rejection of valid evidence for the opposed theory as well as the acceptance of assumptions of doubtful worth in the theory favored. When the validities in the two conflicting hypotheses are reëxamined in the light of advances in knowledge, they prove adjustable into a third and inclusive concept involving both contagion and hereditary susceptibility.[1g,5,12]

Similarly, in the epoch before the infection and its mode of spread became the central idea in its epidemiology, numerous studies of familial aggregation in poliomyelitis were interpreted as indicating an hereditary influence.[19,23,27]

As Hirsch[12] pointed out in a discussion of hereditary predisposition in leprosy, "The best ground on which to try this question is obviously afforded by the small, closely circumscribed, and therefore easily surveyed leprosy spots, with a fixed population subject to no change, where the state of health in the several families may be learned with the least possible trouble and followed with certainty through a long series of generations." A number of rural outbreaks of poliomyelitis, in which the conditions conform to those prescribed by Hirsch as best suited to the study of familial predisposition, have been investigated.

Taylor[25] reported an outbreak of 7 cases of poliomyelitis in Cherryfield, Maine, in 1896: the second case was a brother of the first, and the third case was a cousin of the first and second cases.

Wickman[27] observed 54 cases during an extensive outbreak in Stockholm in 1899: "In several families 2 or 3 cases were observed among cousins, brothers and sisters."

Blackhall, reporting 6 cases in Queanbergen, New South Wales, in 1903–1904, observed that 2 cases were sisters and 2 were cousins.

In an isolated rural outbreak observed by the author in Concord, Vermont, in September and October, 1922, a non-epidemic year, there were upwards of a dozen mild illnesses suspected as being abortive poliomyelitis, and 5 paralytic cases, all related, as shown in Chart 2.

A rural outbreak in Newbury, Vermont, in 1923, reveals a similar relationship between all paralytic cases. No circumstance in common could be established except residence in the same locality (but not on adjacent farms) and blood relationship between cases.

It was in this outbreak that the 1 instance of conjugal poliomyelitis (already discussed) occurred. For a number of years this circumstance was held as contrary to the idea of familial susceptibility, until kinship

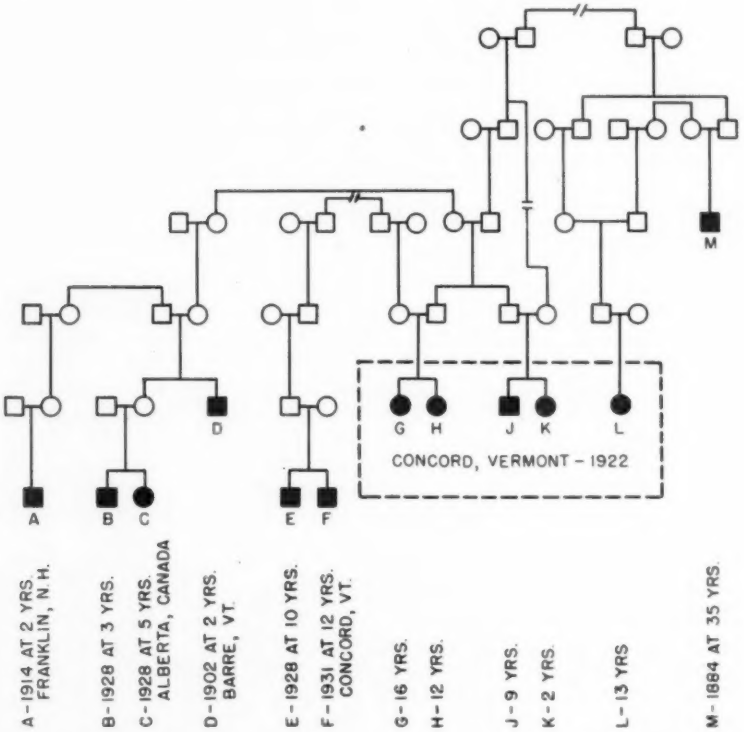
M
D
G H J K L
CONCORD, VERMONT – 1922
A B C E F
A – 1914 AT 2 YRS. FRANKLIN, N.H.
B – 1928 AT 3 YRS.
C – 1928 AT 5 YRS. ALBERTA, CANADA
D – 1902 AT 2 YRS. BARRE, VT.
E – 1928 AT 10 YRS.
F – 1931 AT 12 YRS. CONCORD, VT.
G – 16 YRS.
H – 12 YRS.
J – 9 YRS.
K – 2 YRS.
L – 13 YRS
M – 1884 AT 35 YRS.

CHART 2.—Relationship between 5 cases of paralytic poliomyelitis occurring in an isolated rural outbreak of the disease and relationships to other cases at other times and in other localities.

between husband and wife came to light through tracing relationship of a later case in another part of the state to both of them (Chart 3).

An isolated rural outbreak in New York State in 1940, reported by Langmuir,[16] is of particular interest in the present connection because of the fact that through virus detection studies carried out by McClure, the pattern of virus distribution in the outbreak was actually determined.

Following this outbreak, the virus of poliomyelitis was detected in a number of members of each of 4 families, usually the majority, by inoculation of fecal specimens into monkeys. In 3 of the families, paralytic cases occurred and in all 4 families involved there were non-paralytic or milder suspected abortive illnesses at the same time; while some of the members of each family remained well. The virus was detected not only in paralytic, non-paralytic and suspected abortive cases, but in healthy members of the families as well.

As revealed by a subsequent investigation with Drs. Langmuir,

McClure and Korns, relationship was established between 2 of the families in which 4 of the paralytic cases occurred, and probable relationship between these and the fifth paralytic case. All these cases carried the same family name, which probably traced back to 2 brothers who originally settled in the community but whose family lines have remained apart.

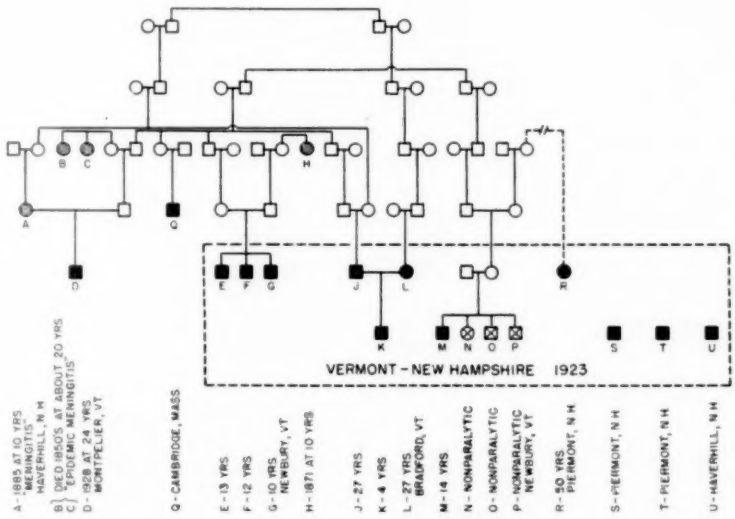
VERMONT – NEW HAMPSHIRE 1923
A - 1885 AT 10 YRS MENINGITIS HAVERHILL, N H
B) DIED 1850'S AT ABOUT 20 YRS
C) EPIDEMIC MENINGITIS
D - 1928 AT 24 YRS MONTPELIER, VT
Q - CAMBRIDGE, MASS
E - 13 YRS
F - 12 YRS
G - 10 YRS NEWBURY, VT
H - 1871 AT 10 YRS
J - 27 YRS
K - 4 YRS
L - 27 YRS BRADFORD, VT
M - 14 YRS
N - NONPARALYTIC
O - NONPARALYTIC
P - NONPARALYTIC NEWBURY, VT
R - 50 YRS PIERMONT, N H
S - PIERMONT, N H
T - PIERMONT, N H
U - HAVERHILL, N H

CHART 3.—Rural outbreak of 7 cases of paralytic poliomyelitis in Vermont and 4 cases in New Hampshire occurring at the same time, showing relationships between cases and to other cases at other times and in other localities.

There are many imperfections in any statistical approach to a study of familial susceptibility to disease, chief among which are discrepancies in the data which are available or which can be obtained with reference to the past. The ideal would include not only extensive genealogies of cases and controls, but a complete record of the occurrence of the disease in them. Furthermore, in contrast with studies of conditions manifest at birth, such as albinism, completeness would require that all individuals in the genealogies had lived beyond the age at which the disease usually manifests itself, or that exposure to the environmental factor by which susceptibility is expressed has occurred. And to do all this would involve the recording of the various pertinent events as they occurred over long periods of time.

Except in hereditary conditions which are manifest early or are outstanding, as for example hemophilia or albinism, the questionnaire-history method of collecting the data is highly unsatisfactory, for the reason that a sufficient span of time, memory or knowledge is not often encompassed by the informant. The average person not only has a more or less restricted acquaintance with his family line, but an even more discrepant knowledge of the occurrence of disease in its members. The difficulties in ascertaining the facts concerning disease in relatives

of patients other than members of the same household by the questionnaire-history method are impressed on one by the frequency of a prompt "No" to the inquiry as to occurrence in relatives and the turning up, on further investigation, of even a number of cases in relatives.

Paralytic poliomyelitis has certain features which make it possible to pursue the study of its familial occurrence far beyond the simple history-questionnaire procedure. In the first place, since it is a relatively uncommon disease, familial concentration is more easily detected than in a fairly common disease. Furthermore, it occurs for the most part at an early age, so that it is not necessary to await the completion of a life span for histories of individuals, and thus the investigation can be contemporary to a greater extent than is the case in studies of disease in older persons, as for example cancer. Finally, paralytic poliomyelitis leaves permanent and easily recognized effects by which it can be identified in the individual at any time subsequent to the attack.

The following family history, not in itself particularly suggestive of any familial tendency, well illustrates the failure of the history method and success by devious methods which are made possible by the characteristics of poliomyelitis.

Three children in 1 family had poliomyelitis in Cambridge, Mass., in 1931. A few years later, when making familial studies, this family stated that there were no cases of poliomyelitis in any known relatives, no matter how distant. But on pressing the question as to lameness or crippling disease of any sort in any member of the family, the reply was, "Nothing like infantile paralysis." It was then stated that there were 2 cousins who had been crippled many years ago—the family could not remember the year. But when it was learned that these cousins lived in Cherryfield, Maine, and the family was told of the outbreak of poliomyelitis there in 1896, they were able to verify the guess that the 2 cousins became crippled that summer.

The names of the individuals coincided with the initials of 2 of the cases in "An Epidemic of Poliomyelitis" reported by Dr. J. Madison Taylor.[25] In this report a case was also found in a third cousin, never mentioned by the family. Thus 3 cases were verified where none was obtained by the history method.

By 1942, something had recalled to the mother of the Cambridge family that still another cousin in Cherryfield had also had the disease about the same time—was still living there and was lame. In reply to an inquiry, this individual wrote on January 12, 1942: "I did not have the disease, although my cousin may have so considered, as I have a shortening of the right leg due to osteomyelitis at age 15, about three years after the infantile paralysis epidemic."

The details of this negative history are included to show that the building up of family histories has not been a matter of including any lameness thought to be poliomyelitis, but to illustrate how, through additional verification of diagnosis, poliomyelitis has been included and lameness due to other diseases equally well excluded.

Summary. In the light of widespread dissemination of the virus, which can be held as a more or less fixed variable in the distribution of the paralytic disease, the familial aggregations of paralytic poliomyelitis reviewed in this paper are indicative of familial susceptibility to paralysis. This conclusion is warranted not so much because of the

significance of these sets of observations taken singly, but because they are found with the relative frequency which would be expected if a familial factor were operative.

The observations included here do not represent so much a preplanned study of familial susceptibility to poliomyelitis as familial aggregations of the disease which have been encountered in the course of a general epidemiologic study. The idea of assembling such data has resulted more largely from the impressiveness of the experience of returning to a locality to see a child whose parent had been seen a good many years before as a child with the same disease. The familial aggregations reported here are enough to warrant the inclusion of similar studies in systematic epidemiologic investigations.

The intensity with which hereditary susceptibility is exhibited in the distribution of the disease, as indicated by these studies, clearly is not sufficient to afford any basis in itself for control measures, but rather forms a background for a study of the nature of individual susceptibility to paralysis; and suggests that the search for predisposing causes, long centered on immediately precedent environmental circumstances such as sanitary conditions, stresses, strains and injuries, nutrition, and so on should include conditions inherent in the constitutional makeup of the individual.

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PHYSIOLOGY.

PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA
SESSION OF JANUARY 20, 1942

The Metabolism of Alanine Containing C^{13}. S. Gurin and D. Wright Wilson (Department of Physiological Chemistry, University of Pennsylvania). C^{13} has all the chemical properties of ordinary carbon but its atomic mass is slightly greater. For this reason, it may be detected by a mass spectrometer whenever it is in sufficient concentration. When a compound containing sufficient C^{13} is fed, the C^{13} may be followed through the body, a procedure which has been called the feeding of tagged elements. Following tagged elements through various compounds in the body may show what changes the fed materials undergo during metabolism. The method constitutes a very valuable aid in the study of intermediary metabolism.

We have synthesized alanine containing excess C^{13} in the carboxyl group. When this alanine is fed to a phlorhizinized rat, a large amount of glucose is excreted which has been thought to come directly from the alanine. However, the glucose contains a very small amount of C^{13}. This proves that most of the sugar of the urine is formed not from the alanine fed but from other sources in the body.

Respiratory Reflexes from the Limbs. J. H. Comroe, Jr. and C. F. Schmidt (Department of Pharmacology, University of Pennsylvania). Since Nielsen has shown that muscular exercise can increase greatly minute volume of respiration without any detectable change in arterial CO_2, O_2 or pH, we attempted to demonstrate the presence of (*a*) chemoreceptors or (*b*) stretch receptors in the limbs capable of stimulating respiration reflexly. Repeated and varied attempts to discover chemoreceptors activated by physiologic stimuli all failed, although reflex hyperpnea was produced by injection into the femoral artery of chemical substances in concentrations beyond physiologic limits (acids, KCl, phosphate buffers pH 6.4, 6.1).

However, movements of the limbs in anesthetized dogs regularly produced hyperpnea. In 11 dogs, active movements of hind limbs (produced by anterior root stimulation) increased respiration 62%, and in 17 dogs passive movements increased respiration 50%. Mere flexion of the limbs often produced hyperpnea equal to that following rapid, alternating flexion and extension. In 70% of 33 normal human subjects, passive movements of one leg produced an increase of more than 30% in respiratory minute volume.

These changes were shown to be due to reflexes for denervation of the limbs in dogs abolished the responses even though the artery and vein were intact, and, in man, spinal anesthesia in 5 subjects greatly reduced or abolished the hyperpnea. In dogs, the receptors for this

reflex lie in or around the joint for procainization of this region abolished the response even though the leg muscles were still intact. It is probable that a large portion of the hyperpnea of muscular exercise in man is referable to reflexes arising from proprioceptors in the joints.

Dark Adaptation of Single Visual Nerve Cells. H. K. HARTLINE and R. MCDONALD (Johnson Foundation and Department of Ophthalmology, University of Pennsylvania). The ability of the eye to adjust itself to widely different levels of illumination is a property inherent in its receptor elements. This property has been studied by direct observation of the discharge of nerve impulses in single optic nerve fibers from the eye of *Limulus*. During prolonged steady illumination the frequency of the discharge initiated by a visual receptor cell declines from its initially high value to a lower level. This may be ascribed in part to the bleaching of the photosensitive substance primarily responsible for sensitivity to light. The sensitivity of the receptor does not decline to zero, however, and impulses continue to be discharged at a uniform rate during very long exposure to light. This may be ascribed to a "back" reaction which keeps the supply of photosensitive material replenished. When the light is turned off this "back" reaction, unopposed by photolysis, leads to a recovery of sensitivity of the receptor cell. This recovery during dark adaptation may be followed by recording at intervals the discharges of impulses elicited by a short test flash of light. The recovery of sensitivity is initially rapid and approaches the dark adapted level asymptotically. The higher the intensity of the preceding light adaptation, or the longer its duration, the greater is the loss of sensitivity measured at the beginning of dark adaptation, and the slower the rate of recovery. Quantitatively the curves resemble those obtained by subjective measurements on human subjects.

Cell Division.* WARREN H. LEWIS (Wistar Institute of Anatomy and Biology, University of Pennsylvania). The *contractile tension* which protoplasm automatically exerts when in the gel state plays important rôles in cell division. Active cells have a superficial gel layer and a less viscous endoplasm. The gel layer, the motor organ of the cell, undergoes local changes of viscosity and corresponding local changes of contractile tension which may result in alterations of cell form, in locomotion, or in cell cleavage. During prophase, pseudopodia retract and cells tend to become spherical. Increased viscosity (increased contractile tension) of the gel layer of pseudopodia accounts for their retraction. The spherical condition is due to a uniform viscosity of the gel layer. During metaphase, the cell becomes approximately bilateral with the chromosomes in the equatorial plane. As the chromosomes move to the poles, the cell flattens in the equatorial region, due to increased contractile tension of a broad equatorial band of the gel layer which has become more viscous. Then follows the equatorial constriction, which deepens

* A motion picture demonstration.

until the cell is divided except for a connecting stalk. The constriction is due to the contraction of a narrow equatorial band of the gel layer which has become still more viscous. Changes of viscosity of cytoplasm and nucleus are probably responsible for other visible phenomena of cell division, such as the appearance of chromosomes in prophase, their movements in late prophase, metaphase and anaphase, and bleb formation during anaphase. The factors responsible for local changes of viscosity, the formation of the gel layer, and the less viscous condition of the endoplasm are unknown.

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